Roles of Corneal Epithelial Ion Transport Mechanisms in Mediating Responses to Cytokines and Osmotic Stress

Peter S. Reinach, José E. Capó-Aponte, Stefan Mergler, and Kathryn S. Pokorny

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OVERVIEW

Normal vision depends, in part, on the combined refractive powers of the cornea and crystalline lens to permit adequate focusing of light onto the retina. Such refractive function requires that the cornea remain transparent, a requirement that is met provided that corneal hydration, i.e., deturgescence, is maintained within specific physiological limits. Maintenance of corneal deturgescence is reliant upon coupled ion and fluid transport activities within the epithelial and endothelial layers. Net ion transport activity offsets the natural tendency of the corneal stroma to imbibe fluid from the anterior chamber, thus keeping the cornea transparent (1–5). Although most of the ion transport activity involved in maintaining corneal deturgescence is contingent upon ion transport processes localized in the corneal endothelial layer, corneal epithelial ion transport activity plays a fine-tuning role in maintaining corneal deturgescence during exposure to environmental challenges (6) (Fig. 1). Only under maximally stimulated conditions is the epithelial-side fluid transport rate able to increase sufficiently, i.e., to approximately 25% of the endothelial-side fluid transport rate (7). This realization has prompted a host of studies concentrated on characterizing receptor-mediated regulation of corneal epithelial active ion transport.
Results of these investigations are relevant for identifying clinical strategies for improving corneal renewal and transparency, and for optimizing the cornea's refractive properties.

The cornea comprises three very different tissue types. In humans, there is an approximately 50\(\mu\)m-thick outer epithelial layer that faces the tears; an approximately 10\(\mu\)m-thick inner endothelial layer that is exposed to the aqueous humor contained in the anterior chamber \((8)\); and an intermediate 450\(\mu\)m-thick stromal layer, comprising orthogonally arranged collagen bundles within a glycosaminoglycan-containing ground substance. Distributed within this matrix are keratocytes, which secrete the macromolecules that form the ground substance \((9)\). Not only the organization, but also the embryonic origins of these tissues, differ greatly from one another. The epithelium is derived from ectoderm and is 5 to 7 cell-layers thick. The uppermost epithelial layers, which are adjacent to the tears, form tight junctions of relatively high electrical resistance, thus providing the crucial barrier function of the cornea. The endothelium and keratocytes are derived from neural crest cells and mesenchymal tissue, respectively. The endothelium comprises a single-cell layer, with tight junctions of low electrical resistance—a property reflected by the endothelium’s much higher solute and greater ionic permeabilities than exhibited by the epithelial layer \((10)\). Epithelial layer ion transporters elicit coupled net salt and fluid movement outwards from the stroma into the tears, whereas endothelial transporters mediate salt and fluid translocation inwards to the anterior chamber. The endothelial layer fluid-transport rate exceeds that of the epithelial side, as evidenced by the fact that net solute transport into the anterior chamber is greater than that into the tears. Therefore, under normal conditions, the contribution of the corneal epithelium to maintenance of transparency and deturgescence is much less than that of the endothelium. Nevertheless, the barrier function of the epithelium is critical for
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protecting the cornea and other intraocular tissues from the damage imposed by exposure to environmental insults.

This review describes the involvement of ion transporter and channel activity in mediating cytokine receptor control of responses required for corneal epithelial function and renewal. Accordingly, a description is first provided of the mechanisms mediating in this tissue osmolyte transport and cell-volume regulation. With this background, it is then possible to appreciate how changes in ion transport and channel activity contribute to cytokine-mediated control of corneal epithelial cell renewal and transparency. Finally, we discuss new evidence that transient receptor potential (TRP) protein superfamily expression and function activate cytokine receptor-linked signaling events inducing responses needed for the maintenance of these corneal epithelial functions.

INVOLVEMENT OF ION TRANSPORT MECHANISMS IN MEDIATED RECEPTOR CONTROL OF CORNEAL EPITHELIAL CELL RENEWAL AND VOLUME REGULATION

Identification of receptors that mediate control of corneal epithelial ion transporter function has been partially elucidated. There are adrenergic (11), serotonergic (12), and cholinergic (13) receptors that contribute to the control of ion transport activity. Regulation of ionic transport in frog corneal epithelia has been demonstrated to be under adrenergic control (14–16). Serotonergic control is shown by the finding that neural serotonin stimulates chloride transport in rabbit corneal epithelia (12). Endogenous cholinergic agonists are able to stimulate active ionic transport in rabbit corneal epithelia. Similarly, cholinergic receptors mediate regulation of epithelial cell proliferation (13, 17). Vital for perpetuation of corneal epithelial function are a host of cytokines that are released into the tears and anterior chamber from the epithelium and accessory tissues of the anterior ocular surface. Cytokine expression is critical for inducing control of proliferation and cell migration through stimulation of cognate receptors (18). Accordingly, control of cell proliferation and migration by cytokines and neuronal agonists is required for synchronization of the corneal epithelial renewal process and preservation of corneal epithelial functions. The uppermost epithelial layers are continuously being lost (into the tears during their terminal differentiation) and replaced, the latter process being necessary for maintenance of corneal transparency. Active ion transporters and channels are components of a myriad of cell signaling pathways that mediate cytokine receptor control of this renewal process (19, 20). Understanding of these events, coupled with the requirement for ion transport activity in maintaining corneal epithelial transparency, has prompted further studies into the mechanisms by which epithelial receptors elicit control of corneal epithelial renewal through stimulation of ion transport activity.

Synchronized epithelial renewal is essential for the maintenance of the cornea’s multilayer integrity and function. Renewal of the upper epithelial layer preserves tight junctional resistance. In addition, epithelial renewal sustains receptor-mediated control of net ion transport, which is required for receptor modulation of epithelial membrane and tight junctional permeability (21). Should epithelial layer renewal become compromised, net ionic fluxes will decline as result of decreased ionic pump function, loss of membrane permselectivity, and decreased tight junctional electrical resistance (22).
Under physiological conditions, cellular losses due to terminal differentiation are countered by replacement with younger cells located in the inner cell layers. This renewal process ensures continuation of normal epithelial ion transport activity. Consequently, epithelial receptor-mediated control of renewal is required for the maintenance of corneal transparency and barrier function.

Even though the contribution of epithelial ion transport activity to deturgescence is much less than that of its endothelial counterpart, the epithelial component is required for the preservation of the integrity of the epithelial layers during exposure to anisosmotic stresses. Anisosmotic insults to the cornea frequently occur during activities of daily life, e.g., swimming or bathing, as well as from contact lens wear and ocular diseases such as dry eye syndrome (DES), as result of which, afflicted individuals experience chronic exposure to hypertonic tears. The physiological disturbances induced by such anisosmotic stresses are, very likely, counteracted by regulatory volume-response activation, such activity having been described in cultured corneal epithelial cells. Two different types of regulatory volume activations have been described for such an in vitro system. Exposure to a hypotonic challenge induces regulatory volume decrease (RVD) behavior, which acts to restore isotonic cell volume (23, 24). Such cell volume restoration is partially due to increases in K⁺ and Cl⁻ membrane permeability, which results in KCl egress coupled to fluid loss. In human corneal epithelial cells, this regulatory response brings about complete recovery of isotonic cell volume within minutes of onset of the hypotonic stress. In contrast, cell exposure to hypertonic challenges, which simulate increased tear-film osmolality in DES (25), induces another type of regulatory volume response, referred to as the regulatory volume increase (RVI) (24, 26). Regulatory volume increase behavior restores the cell’s isotonic volume through stimulation of ion and solute influx transport mechanisms, which mediate a rise in intracellular osmolyte content coupled with net fluid influx. Even though the RVI response is somewhat slower than the RVD response, corneal epithelial cells are able to achieve complete restoration of their isotonic volume following anisosmotic stress. In corneal epithelial cells, there is some evidence that specific receptors sense changes in osmolarity and activate a unique array of signaling pathways. These signaling pathways have some features in common to those linked to the cytokine receptors that mediate control of the corneal epithelial responses—proliferation and migration—required for renewal (27). This commonality—coupled with results from recent studies of other tissues, showing that cell volume regulation is requisite for proliferation and migration—suggests the importance of ion transport regulation in the maintenance of corneal epithelial function (28).

There is emerging evidence that receptor-mediated control of ion transport activity is requisite for corneal epithelial renewal (27, 29). This requirement is self-evident, since parent cell volume must increase to accommodate rises in genomic and cytoplasmic content prior to karyokinesis and cytokinesis. Similarly, changes in cell volume are requisite for cell migration, as this process involves repeated, coordinated leading-edge cytoplasmic volume extension, with retraction at the opposite pole. Because changes in ion transport activity underlie cell volume regulation, cytokine-induced control of renewal is, thus, dependent upon modulation of ion transport mechanisms. For this control to occur, corneal epithelial-induced cytokine expression of the corneal epithelium and accessory ocular tissues must be synchronized and manifested at appropriate times to alter cell volume, and to allow cell-cycle progression and migration to occur unperturbed. Numerous cytokines are involved in regulating these processes (18), some of
which are needed to elicit control of proliferation and migration, while others affect rates of differentiation. Such controls occur through a host of cell-signaling pathways, each of which is specific for one of the cognate receptors. Any particular cytokine can elicit control of a variety of responses by activating different pathways within a cell-signaling network. The mitogen-activated protein kinase (MAPK) cascade is a superfamily of cytokines that mediates this type of exquisite control. Mitogen-activated protein kinase cascades have three different parallel pathways: the extracellular signal-regulated kinase (ERK); the p38; and the c-jun N-terminal/stress-activated protein kinase (JNK/SAPK) pathways. In corneal epithelial cells, different stimuli selectively activate specific receptors linked to one or more of these three different pathways. Cytokines that mediate increases in proliferation induce a response through stimulation of the ERK (i.e., p44/42) pathway, whereas those involved in increasing cell migration act through the p38 MAPK pathway (30–34). Stressors, such as anisosmotic challenges or apoptosis-inducing agents, e.g., ultraviolet light, activate the JNK/SAPK pathway (29, 35). Furthermore, the ability of a cytokine to elicit a particular response can be modulated by crosstalk between different branches of a cell-signaling network that is linked to different responses. Such

**Fig. 2.** Mitogen-activated protein kinase (MAPK) pathways in corneal epithelial cells. Diagram depicting the three MAPK pathways (p38, extracellular signal-regulated kinase [ERK], and c-jun N-terminal/stress-activated protein kinase [JNK/SAPK]) and their respective cellular function in corneal epithelial cells. The broken arrow indicates phosphatase-mediated crosstalk control of growth factors between p38 and ERK MAPK signaling. This control modulates the magnitude of growth factor-induced increases in corneal epithelial cell migration and proliferation, both of which are required for wound healing or apoptosis.
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