Pulmonary epithelial barrier function: some new players and mechanisms

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Brune K, Frank J, Schwingshackl A, Finigan J, Sidhaye VK. Pulmonary epithelial barrier function: some new players and mechanisms. Am J Physiol Lung Cell Mol Physiol 308: L731–L745, 2015. First published January 30, 2015; doi:10.1152/ajplung.00309.2014.—The pulmonary epithelium serves as a barrier to prevent access of the inspired luminal contents to the subepithelium. In addition, the epithelium dictates the initial responses of the lung to both infectious and noninfectious stimuli. One mechanism by which the epithelium does this is by coordinating transport of diffusible molecules across the epithelial barrier, both through the cell and between cells. In this review, we will discuss a few emerging paradigms of permeability changes through altered ion transport and paracellular regulation by which the epithelium gates its response to potentially detrimental luminal stimuli. This review is a summary of talks presented during a symposium in Experimental Biology geared toward novel and less recognized methods of epithelial barrier regulation. First, we will discuss mechanisms of dynamic regulation of cell-cell contacts in the context of repetitive exposure to inhaled infectious and noninfectious insults. In the second section, we will briefly discuss mechanisms of transepithelial ion homeostasis specifically focused on the role of claudins and paracellular ion-channel regulation in chronic barrier dysfunction. In the next section, we will address transepithelial ion transport and highlight the role of Trek-1 ion channels in epithelial responses to lung injury. In the final section, we will outline the role of epithelial growth receptor in barrier regulation in baseline, acute lung injury, and airway disease. We will then end with a summary of mechanisms of epithelial control as well as discuss emerging paradigms of the epithelium role in shifting between a structural element that maintains tight cell-cell adhesion to a cell that initiates and participates in immune responses.

lungs; epithelial barrier; permeability; transport; ions

THE LUNG EPITHELIUM IS A MUCOSAL SURFACE composed of ciliated cells, mucous-producing cells, and undifferentiated basal cells that forms the interface between the lumen and the parenchyma from the nasal passage to the alveoli. It is the initial site of contact for all inspired substances and therefore acts as both a physical and an immunological barrier that protects the subepithelial tissue. The physical barrier, comprised of the mucociliary apparatus, secreted antimicrobial substances, and the intercellular junctions, prevents inhaled toxins and pathogens from contacting the subepithelial tissue. The mucociliary apparatus traps foreign particles and removes them from the respiratory tract. Defects in this apparatus can lead to recurrent infections, as seen in patients with cystic fibrosis (104), chronic obstructive pulmonary disease (COPD) (14, 79), and ciliary dyskinesia (18, 19). Secreted antimicrobial substances including enzymes, protease inhibitors, and antimicrobial peptides further contribute to the immune response of the epithelial cell (134). Epithelial secretions contain enzymes such as lysozyme that have antimiicrobial effects against both Gram-positive and Gram-negative bacteria (40, 76) as well as protease inhibitors, including serine protease inhibitor, secretory leukocyte protease inhibitor, and elafin, which reduce the effects of proteases that are produced by pathogens. In addition, surface antimicrobial peptides like human β-defensins further protect against numerous pathogens, including bacteria and viruses (116, 176).

The intercellular junctions form adhesive forces that connect neighboring cells and separate the external environment from the subepithelial tissue. There are three main types of intercellular junctions: the tight junctions, the adherens junctions, and the desmosomes (137). The tight junctions are located more apically compared with the other junctions and are multiprotein complexes that consist of transmembrane proteins, including claudins, occludins, and junctional adhesion molecules, as well as scaffolding proteins like the zonula occludens (ZO). These junctions form a continuous circumferential ring around the epithelial cells, which regulates the paracellular transport of...
ions and certain molecules (63, 157). The adherens junctions, composed of E-cadherin and several proteins from the catenin family, including α-catenin, β-catenin, and p120, anchor the actin cytoskeleton and mediate cell-to-cell adhesion (41, 63, 78). Together, the tight and adherens junctions form the apicoadherens junctions, which separate the apical and basolateral membranes and block the movement of integral membrane proteins between surfaces to maintain epithelial cell polarity (169). The desmosomes, adhesive junctions located on the lateral cell membrane, link to the intermediate filaments and help protect against mechanical stress. Inhaled toxins such as cigarette smoke damage these intercellular junctions, resulting in a leaky, hyperproliferative, and abnormally differentiated epithelium (22, 68, 73, 130, 150, 191).

It is increasingly recognized that the epithelium helps to maintain homeostasis in the lung, and disruption of the epithelial barrier can lead to the development of inflammation and diseases such as COPD and acute lung injury (ALI). Moreover, novel studies demonstrating that restoration of the epithelial barrier through transfer of mitochondria from bone marrow-derived stromal cells to epithelial cells can protect against ALI further establish the importance of the epithelial barrier (77). In this review, we will further discuss how the airway epithelium dynamically regulates its response to luminal stimuli by altering ion transport and paracellular permeability.

The Lung Epithelium: Insights into the Role of Regulation of Paracellular Permeability in Epithelial Signaling

The role of cell-cell adhesion in modulating epithelial signaling pathways. The integrity of the physical barrier and epithelial cell polarity affect the interaction of cytokines and growth factors with their receptors, thereby modulating the immunological response of the epithelium environmental stimuli. In this respect, the epithelium also acts as an immunological barrier. Subtle changes in paracellular permeability can impact ligand interaction with its receptor. Moreover, certain cytokines are prevented from interacting with their cytokine receptors because of segregation to different sides of the apicoadherens junctional complex. Damage to the epithelium can affect the polarized distribution of cell surface receptors, allowing for increased receptor-ligand interaction and the activation of signaling transduction cascades and secretory activity (73, 171, 200). One specific example of such regulation is the epidermal growth factor (EGF), which regulates migration, proliferation, and differentiation following injury and enhances epithelial repair (186). In normal human airways, EGF receptor (EGFR) expression is limited and isolated to the basolateral membrane (187); however, expression is increased in response to cigarette smoke (165, 188), mechanical stress (139), and inhaled noxious substances like bleomycin (110). In a homeostatic state, the EGF ligand is located on the apical surface and therefore separated from its receptor on the basolateral surface. Moreover, the apically located growth factor heregulin is separated from its receptor erbB2/3 (also known as HER2/3), which is located on the basolateral membrane. These growth factors, therefore, only have access to their receptors when the epithelium is damaged, allowing for rapid targeted repair of the epithelial injury (200). The binding of heregulin to the erbB2/3 receptor leads to cell proliferation, differentiation, and mucous secretion (193, 201, 209), which allows the damaged epithelium to protect and repair itself. Therefore, although the epithelium expresses both the growth factors and their receptors, it can regulate the interaction such that the effects occur only at times of injury, and one mechanism by which the epithelium does this is by regulating paracellular permeability.

Dynamic regulation of paracellular permeability. The intercellular junctions do not statically resist entry to all substances but act as dynamic structures that can open or close in response to both physiological and pathological stimuli (157, 167). By tightening cell-cell adhesion in response to mechanical stimuli such as shear stress or chemical environmental exposures such as inhaled particulate matter, the epithelia dynamically regulates receptor-ligand interactions (Fig. 1) (171). For example, airflow over the epithelium during breathing creates shear stress, which can lead to changes in barrier properties. Low, physiological levels of shear stress are generated during normal tidal volume breathing but can increase by several times during exercise, coughing, and bronchospasm. Exposure to shear stress leads to actin rearrangement and barrier enhancement (173). In addition, in response to both physiological levels of shear stress as well as pathological stimuli like particulate matter, pollution formed from the mixture of solid particles and liquid droplets found in air, there is increased membrane localization of septin 2 that decreases paracellular permeability by altering cortical actin rearrangement (171). Septin 2 is a member of the family of GTP-binding proteins that function in cytoskeletal arrangement and cell polarization. Membrane localization of septin 2 allows for increased interaction with actin, which regulates the rearrangement of cortical actin and enhances barrier function. Physiological levels of shear stress also lead to a selective decrease in the abundance of the apical water channel aquaporin 5 (172, 173), which helps to modulate the paracellular permeability in response to the stress. Aquaporin 5 stabilizes the microtubules (172) and antagonizes cell-to-cell adhesion (173), thereby affecting paracellular permeability. Thus the epithelium is able to dynamically regulate its response to experienced luminal stimuli.

In summary, the airway epithelium is the first line of defense and prevents inspired substances from contacting the subepithelial tissue. The epithelium acts as both a physical and immunological barrier that continuously responds to both physiological and pathological stimuli in the environment and dynamically regulates its barrier in response to these stimuli. The apicoadherens junctions separate the apical and basolateral membranes, and therefore membrane integrity can affect cell signaling and the defenses mounted in response to environmental exposures. By tightly coordinating epithelial responses to environmental exposures and altering epithelial signaling, secreted products, and ion and water flux in response to stimuli, the epithelium is able to provide a dynamic barrier at the interface between the lung parenchyma and the luminal environment. Noxious stimuli such as chronic tobacco exposure, infections, particulate matter, and air pollution can lead to a breakdown in this regulatory response, leading to chronic epithelial activation, which can further propagate the damage, resulting in a leaky barrier. In the following sections, we will discuss in more detail how the tight junction proteins, ion transport, and EGFR contribute to the dynamic epithelial barrier.
The Lung Epithelium: Insights in Ion Transport Across the Barrier

Interactions between tight junctions and ion transporters to maintain lung fluid balance. Control of airspace fluid volume and composition in the alveolus is primarily determined by transcellular ion transport, which requires a polarized epithelium with intact tight junctions. However, paracellular ion, macromolecule, and water transport through intact tight junctions also makes a significant contribution to lung fluid balance (115, 199). Furthermore, the effects of both bacterial and viral infections are quite complex, with data suggesting that a multitude of ion channels are altered in response to infection, and there is potential for creation of new channels by a host of viruses, termed viroporins. The detailed discussions of these effects are beyond the scope of this review. During the resolution of pulmonary edema, clearance of fluid from the airspaces is largely dependent on a transepithelial sodium concentration gradient driven by Na⁺/K⁺-ATPase. Apical sodium conductance through epithelial sodium channel (ENaC) is central to fluid clearance, but chloride and potassium channels also make important contributions (13, 37, 98). Apical-to-basolateral chloride transport may be particularly important because the maximal rate of sodium and water transport from the airspaces appears to be limited by the concomitant transport of chloride in many studies (9, 43, 122). Because experimental data suggest that a sizable fraction of transepithelial chloride transport occurs through the paracellular route in the alveolar epithelium (87), regulated paracellular ion transport could influence alveolar fluid clearance rates by this mechanism. In other words, changes in the selectivity and magnitude of paracellular ion conductance may influence rates of alveolar fluid clearance, either by limiting sodium leak or increasing chloride currents. The mechanisms for the regulation of paracellular transport in the lung and the potential interrelationship with transcellular transport are not fully understood, but recent work has begun to describe some of the major determinants of paracellular permeability in the alveolar epithelium. These studies have also provided some insight into deeper associations between the paracellular and transcellular routes of transport that is the regulation of transcellular transporters by cell junction proteins. One area of interest is the potential association between Na⁺/K⁺-ATPase and tight junction claudins.
Sodium/potassium ATPase as a regulator of cell junctions. Sodium/potassium ATPase is made up of catalytic (α) and regulatory (β) subunits. Additional regulatory subunits of the FXYD family, such as the γ-subunit of Na+/K+-ATPase, may also associate with α/β-heterodimers and affect function. Pump activity in polarized epithelial cells is essential for lung fluid balance, and increased pump activity is associated with increased rates of vectorial fluid clearance across the alveolar epithelium (4, 107). Recent work has uncovered an additional role for Na+/K+-ATPase in tight junction formation and maintenance in epithelia (194). For example, the β-subunit of Na+/K+-ATPase associates with PDZ domain-containing proteins and localizes to tight junctions (109, 141). Although the β-subunit has been reported to function as a cell adhesion molecule, enzymatic pump activity of Na+/K+-ATPase is required for tight junction formation in Madin-Darby canine kidney (MDCK) cells (144). The mechanism for this function appears to be through Na+/K+-ATPase-mediated activation of RhoA signaling and cytoskeletal remodeling at the periphery of epithelial cells. Na+/K+-ATPase may also be required for the maintenance of tight junctions. In monolayers of retinal pigment epithelial cells and in pancreatic ductal cell (HPAF-II) monolayers with established tight junctions, inhibition of Na+/K+-ATPase with ouabain increased paracellular permeability to ions and macromolecules (142, 143). However, the junction-promoting effects of Na+/K+-ATPase may be context or cell-type specific because ouabain has also been reported to increase expression of claudins-4 and -1 in MDCK cells in association with decreased paracellular macromolecule permeability (97). Although the mechanisms for the regulation of tight junction formation and function by Na+/K+-ATPase are not fully understood, it appears that Na+/K+-ATPase can modulate tight junction assembly and function.

Claudins as regulators of paracellular permeability. Differential expression of the claudin family of tight junction proteins is a fundamental regulatory mechanism of paracellular permeselectivity. Claudins are transmembrane proteins that are necessary and sufficient for tight junction strand formation (51). The spatially restricted expression of specific claudins along the course of the nephron exemplifies their permeselectivity function (7). Moreover, point mutations in certain claudins result in clinically important changes in ion transport, and substantial experimental evidence supports a direct role for claudins in charge-selective paracellular ion conductance (6, 102, 195). Claudins on adjacent cells form paracellular pores with an ~3-Angstrom diameter that function as relatively high-conductance, un gated ion channels (101, 145, 197). Claudins also influence paracellular macromolecule transport, and individual claudins appear to convey different properties to this charge-independent, lower-conductance pathway (167, 168). A leading hypothesis is that individual claudin strands within a tight junction establish the ion pore pathway, whereas the persistence or continuity of claudin strands within the membrane influences the macromolecule leak pathway (167). Because tight junctions consist of multiple layers of claudin-based strands that undergo continuous turnover, both conductance pathways can exist simultaneously. Three claudins, claudin-3, claudin-4, and claudin-18.1, are abundantly expressed in the alveolar epithelium although other claudins are expressed at low levels (95).

Lung-specific claudin-18.1 is a requirement for the alveolar epithelial permeability barrier. Claudin-18.1 is of particular interest because it is the only known lung-specific tight junction protein, and it is the most abundantly expressed claudin in type 1 alveolar epithelial cells (AECs) (95). The role of claudin-18 in alveolar epithelial barrier function has recently been examined in knockout (KO) mice. Claudin-18 does not appear to be required for prenatal lung development; however, upon conversion to air breathing, alveolarization is abnormal in claudin-18 KO mice (96). Claudin-18-null mice have a threefold increase in alveolar epithelial permeability to macromolecules. There is a similar increase in macromolecule permeability in cultured primary AECs from KO mice and with siRNA knockdown of claudin-18 in wild-type cells (96). These data indicate that claudin-18 provides a macromolecule permeability barrier in the alveolar epithelium and suggest that the loss of this function results in abnormal alveolar homeostasis and impaired alveolarization. Interestingly, although macromolecule permeability is increased, claudin-18-null mice do not develop pulmonary edema (100). The reason for this may be that alveolar epithelial sodium and chloride transport is increased. The basal alveolar fluid clearance rate in claudin-18-null mice is approximately twice that of wild-type littermates. Examination of the expression levels of ion transporters in whole lung revealed a twofold increase in the β1-subunit of Na+/K+-ATPase and increased pump activity but no change or a slight decrease in the α-, β-, and γ-subunits of ENaC at the mRNA level. Because overexpression of Na+/K+-ATPase results in higher rates of alveolar fluid transport in animal models, the increase in β1-subunit expression may account for most of the increase in alveolar fluid clearance in claudin-18-null mice.

Chloride transport and alveolar fluid clearance in claudin-18-null mice. As mentioned above, sodium transport in the lung can be limited by the cotransport of chloride, so increases in the levels of chloride transporters might be expected to facilitate higher rates of sodium and fluid transport. It is notable that cystic fibrosis transmembrane conductance regulator (CFTR) mRNA expression is increased by approximately fourfold in claudin-18 KO mice, but whether CFTR inhibition decreases alveolar fluid clearance in this context has not been reported. In addition to CFTR, several other regulators of chloride transport show increased expression in the lungs of claudin-18-null mice, including Na-K-2Cl cotransporter (NKCC1), CLC2, and SLC26A9. It is possible that these data point to a larger role for chloride transport in alveolar fluid clearance than has been previously appreciated. For example, chloride-dependent sodium transport into the airspaces appears to be an important mechanism for edema formation during hydrostatic stress (183). In the nephron, chloride-dependent sodium absorption is a mechanism for increased fluid retention and hypertension. This mechanism depends on the coupling of sodium transport to chloride transport via NKCC1. Although NKCC1 transport in a basolateral-to-apical direction is well established, transport in an apical-to-basolateral direction (or bidirectional transport) is possible (183). Therefore, the observed increase in chloride transporters in claudin-18-null mice is consistent with compensation of fluid clearance facilitated by maximizing chloride-limited sodium transport. Further investigation is required, but increased NKCC1 expression in claudin-18-null mice might also support the hypothesis that apical-
Functional implications of increased claudin-4 expression in claudin-18-null mice. Claudin-4 mRNA and protein levels are increased fourfold in claudin-18 KO mice. Claudin-4 plays a distinct role in transepithelial ion transport and junction formation and may partly compensate for the loss of claudin-18. Claudin-4 acts as a relative sodium barrier and is also specifically upregulated during epithelial repair (72, 196, 207). In human and murine lungs, higher claudin-4 expression is specifically upregulated during epithelial repair (72, 196, 207). In mouse studies, the loss of claudin-4 results in decreased alveolar fluid clearance and more severe pulmonary edema with injury (82, 207). These data are consistent with the hypothesis that lung epithelial tight junctions containing high levels of claudin-4 support higher rates of alveolar fluid clearance. This may be because claudin-4 forms high-resistance junctions that limit sodium conductance, resulting in a larger transepithelial sodium gradient. Alternatively, claudin-4 may play a role in the regulation of Na⁺/K⁺-ATPase, but further studies are needed. In claudin-18-null mice, higher levels of claudin-4 may partly explain the observed increase in alveolar fluid clearance.

Functional association between claudins and transcellular ion transport. Taken together, available data suggest a functional association between tight junction claudins and transcellular ion transport. The hypothesis that claudin-4 and Na⁺/K⁺-ATPase have a cooperative role in transepithelial sodium transport in the lung requires further study, but it is clear that claudin-4 is necessary for normal barrier function and maximal rates of fluid clearance. It is also clear that claudin-18 forms a unique permeability barrier in the alveolar epithelium and is a requirement for alveolar homeostasis. The loss of claudin-18 results in a compensatory increase in claudin-4 along with numerous changes in transepithelial ion transporters in association with increased rates of alveolar fluid clearance. The adaptation to chronic barrier leak in claudin-18-null mice may provide novel insights into the mechanisms of alveolar ion and water transport regulation in diseases characterized by epithelial barrier dysfunction.

The Lung Epithelium: The Role of Two-Pore-Domain Potassium Channels in Lung Epithelium

Transcellular ion homeostasis in the lung epithelium. Maintenance of the integrity and the homeostatic properties of the alveolar capillary barrier plays a vital role in pulmonary health and disease (111). Through separation of the air- and blood-filled spaces in the lung, the alveolar epithelium is strategically positioned to interact closely with both environments and to regulate the electrolyte and water content of the alveolar spaces. In this section, we will briefly review 1) the major ion-transport mechanisms regulating the composition of the alveolar lining fluid (ALF) under physiological conditions, and 2) the role of stretch-activated ion channels (SACs) in the development of hyperoxia (HO), mechanical stretch-induced ALI, and acute respiratory distress syndrome (ARDS), with particular emphasis on epithelial barrier function and inflammatory cytokine secretion.

Maintaining tight control over the intra-alveolar environment requires complex synchronization of multiple epithelial ion-transport processes and a dynamic balance between continuous secretion, and reabsorption of Na⁺ and Cl⁻ determines the composition of the ALF (131). When an increase in alveolar fluid absorption is required, as in the immediate postnatal period or in conditions associated with pulmonary edema like pneumonia and congestive heart failure (136, 205), Na⁺ is internalized via apical amiloride-sensitive ENaC channels and cyclic nucleotide-gated cation channels in both alveolar type I (AT I) and type II (AT II) cells (33, 36, 37, 45, 74). Internalized Na⁺ is then secreted into the interstitial space via the basolateral Na/K ATPase (4, 31). To maintain intracellular electroneutrality, apical Cl⁻ absorption occurs mainly via CFTR channels or paracellular pathways (36). In combination, these electrolyte shifts create an osmotic gradient for water molecules to move from the alveolar into the interstitial space either via aquaporin channels or by diffusion (1, 88, 177, 198).

In contrast, when increased production of ALF is required, as part of physiological ALF turnover and aging, to maintain surfactant homeostasis, or in response to infectious or environmental pollution stimuli (67, 81, 106, 120), Cl⁻ anions are actively secreted into the alveolar space mainly via apical CFTR channels, CIC channels, and Ca-dependent Cl⁻ channels (35, 50, 69, 108). An active driving force for Cl⁻ secretion is maintained by basolateral Na/K/Cl cotransporters, K-Cl cotransporters, HCO₃/Cl exchangers (5, 91, 151, 175, 190), as well as basolateral inwardly and outwardly rectifying Cl⁻ channels (10, 69). Na⁺ ions are thought to follow Cl⁻ ions from the interstitial into the alveolar space, but the molecular mechanisms underlying Na⁺ secretion are still not fully understood.

Similarly, basolateral K⁺ channels play an important role in maintaining ALF by creating a favorable electrochemical gradient for Na⁺ absorption and Cl⁻ secretion, by restoring intracellular electroneutrality, and by stabilizing the resting membrane potential (112). A variety of K⁺ channels have been described in both AT I and AT II cells, including voltage-gated (Kᵥ), ATP-dependent (KᵥATP), inwardly rectifying (Kir), Ca²⁺-activated (KᵥCa) K⁺ channels (8, 57), and small- and large-conductance (BK) K⁺ channels (11, 113). In addition to homeostatic processes, K⁺ channels also appear to be involved in epithelial wound healing, cell migration, cell proliferation, and cytokine secretion in vitro and in vivo models of ALI/ARDS (8, 149, 161, 162, 192), and large-conductance BKᵥCa channels may act as oxygen sensors in the lung epithelium (80).
ENaC expression (158); inhibition of \( K_{ATP} \) channels can inhibit ENaC currents (90); activation of \( K_{ATP} \) channels can increase ENaC and CFTR currents (98); and inhibition of \( K_{Ca} \) and \( K_v \) channels can inhibit ENaC and CFTR currents (17, 62). There are numerous limitations in the study of epithelial ion-transport processes; however, more recent approaches employing siRNA and shRNA knockdown of specific ion channels (163), and the broader availability of KO mice deficient in specific ion channels (32, 124) have significantly facilitated research in this field. Nevertheless, conditional KO models for specific epithelial ion channels, where a particular ion channel is absent from AT I or AT II cells only, are not always readily available.

The role of ENaC channels in lung fluid balance. The ENaC channels are composed of four subunits (\( \alpha, \beta, \gamma \), and \( \delta \)) coded for by the \( SCNN1A, SCNN1B, SCNN1G \), and \( SCNN1D \) genes, respectively. The \( \alpha \)-subunit is required to form a functional ENaC channel, and the \( \beta \)- and \( \gamma \)-subunits amplify the activity of the channel. In mice, homozygous KO of the \( \alpha \)-ENaC subunit leads to retention of fetal lung liquid, respiratory distress, and ultimately death (74). In humans, mutations in the ENaC subunit genes \( SCNN1A, SCNN1B, \) and \( SCNN1G \) can lead to autosomal recessive pseudohypoaldosteronism (PHA) characterized by lifelong salt wasting, hyperkalemia, and dehydration (25). Children who have PHA type 1 with mutations in the genes encoding the \( \alpha \)- and \( \beta \)-subunit have an increased volume of fluid in the lungs because of problems with fluid absorption (84), leading to recurrent respiratory problems. There are, however, case reports of preterm infants with a single homozygous mutation in the \( \alpha \)-ENaC subunit who did not have immediate postnatal difficulty with lung fluid clearance, indicating that the \( \alpha \)-ENaC may not be pivotal in the clearance of lung fluid at early stages of lung maturation (75).

Transforming growth factor (TGF)-\( \beta \) has been shown to be important in the regulation of ENaC activity, which has important implications for lung fluid balance. Peters et al. (135) demonstrated that TGF-\( \beta \) can drive the abnormal internalization of the ENaC complex, leading to a reduction in this complex at the lung epithelial cell surface, resulting in reduced sodium and fluid absorption (135). These data suggest a unique TGF-\( \beta \)-dependent mechanism to regulate ion and fluid transport in the lung and potentially a new pathological mechanism of ALI and ARDS.

The role of two-pore-domain potassium channels in ALI and ARDS. The role of SACs in the development of HO and mechanical stretch-induced ALI/ARDS and the consequences of SAC dysregulation on epithelial barrier function and inflammatory cytokine secretion is of particular interest (181, 182). Both in vitro and in vivo studies have demonstrated that mechanical forces can alter transepithelial ion transport, which results in a net decrease in alveolar fluid clearance (50, 146, 204) and is directly associated with an increased length of mechanical ventilation and hospital mortality in patients with ALI/ARDS (70, 181).

The term SACs refers to a variety of ion channels, including \( Na^+ \) channels such as ENaC, \( Cl^- \) channels such as the CIC family, \( Ca^{2+} \) channels such as the transient receptor potential vanilloid family, and numerous \( K^+ \) channels including two-pore domain potassium (K2P) channels, \( K_{ATP} \) and \( K_v \) channels (23, 126). SAC channels are ubiquitously expressed in body tissues including the heart, kidneys, genitourinary tract, gastrointestinal tract, leukocytes, and great blood vessels (179, 202, 203) and regulate a variety of cellular functions including the resting membrane potential, cell volume, gene expression, cell differentiation, and cell-contraction processes (16, 39, 154).

The K2P channel family consists of 15 members and is divided into 6 subfamilies: TWIK, THIK, TREA, TASK, TALK, and TRESK (156). A unique property of K2P channels is that they can be constitutively open, creating so-called K+-leak currents that maintain the negative resting membrane potential of a cell (12, 127), and, in tissues other than the lung, K2P channels have been shown to act as mechanosensors and mechanotransducers (212). For this review, we will focus specifically on the role of TREK-1 in ALI/ARDS.

Regulation and function of TREK-1 channels in models of ALI/ARDS. TREK-1 expression was first detected in mouse, rat, and human AECs (163) but also appears to be expressed in airway epithelial cells (213). Preliminary data show that TREK-1-deficient cultured mouse AECs and human bronchial epithelial cells appear protected from a HO- and TNF-\( \alpha \)-induced loss of transepithelial electrical resistance, suggesting a role for TREK-1 in epithelial barrier (Schwingshackl A, unpublished observations). In addition, stimulation of TREK-1-deficient mouse and human epithelial cells with TNF-\( \alpha \) decreased IL-6 and RANTES secretion and increased in monocytic chemotactic protein-1 (MCP-1) secretion (Fig. 2). In contrast, keratinocyte chemokine/IL-8 secretion remained unchanged between TREK-1-deficient and control cells. Whereas several TNF-\( \alpha \)-activated signaling pathways, including p38 kinase, PKC, and e-JNK1/2/3 were altered in TREK-1-deficient AECs, decreased IL-6 secretion from TREK-1-deficient AECs was related to impaired phosphorylation of the PKC isoform-\( \alpha \) after TNF-\( \alpha \) stimulation, and increased MCP-1 secretion appeared unrelated to the increased phosphorylation of JNK1/2/3 in TREK-1-deficient AECs. The specific molecular signaling mechanisms underlying these alterations in cytokine secretion is presently being investigated.

On the basis of in vitro findings, it was anticipated that TREK-1 KO mice would be protected from HO- and mechanical stretch-induced lung injury. However, exposure of TREK-1 KO mice to 24 h of HO resulted in increased lung injury, as evidenced by gross lung anatomy, hematoxylin and eosin staining of lung sections (increased inflammatory cell
infiltration and hemorrhage), increased lung injury scoring, decreased lung compliance, and increased macrophage and neutrophil accumulation in the bronchoalveolar lavage (BAL) fluid of TREK-1 KO mice (164). Interestingly, exposure of TREK-1 KO mice to HO plus mechanical ventilation resulted in no further injury compared with HO exposure alone (164).

Similar to in vitro studies (163), in vivo models show that HO exposure is a weak stimulus for cytokine secretion, and only HO plus mechanical ventilation increased IL-6, MCP-1, and TNF-α levels in the BAL fluid of both wild-type and TREK-1 KO mice. In accordance with in vitro findings, there were decreased amounts of IL-6 in HO plus mechanically ventilated TREK-1 KO mice (161). However, in contrast to in vitro findings (162), MCP-1 levels were decreased in HO plus mechanically ventilated TREK-1 KO mice, whereas TNF-α levels were unchanged between control and TREK-1 KO mice (164). Therefore, in this model, alterations in cytokine secretion between TREK-1 KO and wild-type mice were unlikely the cause for the observed increase in lung injury in the TREK-1 KO mice. Nevertheless, the contributions of cytokines such as IL-6, IL-1β, and TNF-α to the development of ALI/ARDS in other animal models and in human patients with ARDS are well documented (118, 119). The proinflammatory environment associated with the secretion of these mediators promotes neutrophil-, macrophage-, T cell-, and epithelium-mediated alveolar injury, causes loss of alveolar barrier function, and decreases alveolar surfactant levels (61, 94, 119, 185, 206). Similar to in vitro studies, the specific molecular mechanisms underlying these alterations in BAL cytokine levels in vivo models remain to be determined. In addition, it will be important to investigate the cellular sources for these inflammatory mediators and quantify their relative contributions.

Furthermore, it is of major importance to determine the time course of cytokine secretion in these ARDS models. It is quite possible that, although after 24 h of HO exposure and 4 h of mechanical ventilation BAL IL-6 and MCP-1 levels were decreased in TREK-1-deficient mice, these cytokines were increased at earlier time points and contributed to the observed lung injury. Of note, whereas TNF-α is a known proinflammatory cytokine in the early stages of ARDS, in chronic pulmonary inflammation, it may actually exert anti-inflammatory, beneficial effects and promote physiological wound healing (117).

Interestingly, alveolar barrier function appeared uncompromised in the mouse ARDS model because BAL protein levels were unchanged between control and TREK-1-deficient mice (164). HO alone caused only a minor disruption in alveolar barrier function, whereas the combination of HO and mechanical ventilation resulted in significant BAL protein accumulation in both mouse types. The protective effect of TREK-1 deficiency observed in vitro on TNF-α- and HO-induced loss of barrier function was not seen in the whole animal model. Whether a close interaction between TREK-1 and epithelial tight junction proteins, as seen in cultured AECs (unpublished observations), also exists in vivo remains to be confirmed.

One explanation for the increased lung damage observed in HO-exposed TREK-1 KO mice could be an increase in AT I and AT II cell apoptosis in these animals, as evidenced by TUNEL staining and corroborated by increased poly(ADP-ribose) polymerase-1 cleavage, one of the distal signaling molecules of the caspase cascade (164). Another possible explanation could be a decrease in surfactant protein C detected in TREK-1-deficient mice after prolonged HO exposure, potentially making lungs more vulnerable to HO- and mechanical stretch-induced injury (164). The molecular mechanisms linking TREK-1 deficiency to epithelial apoptosis and decreased surfactant production are presently unknown. As described above, we have observed substantial alterations in p38, PKC, and JNK phosphorylation in TREK-1-deficient epithelial cells after TNF-α stimulation (161, 162), but whether these kinases are involved in apoptosis or surfactant production needs to be determined. Another question that needs to be addressed in future studies is whether K⁺ flux through TREK-1 channels is required for the observed in vitro and in vivo effects or whether TREK-1 could exert K⁺-independent functions and act as a regulatory molecule rather than an ion channel, as reported, for example, in CFTR (159).

In conclusion, whereas in vitro epithelial TREK-1 deficiency appeared protective against the inflammatory stimuli responsible for the development of ALI/ARDS, in an in vivo model of ALI/ARDS, TREK-1 deficiency resulted in increased lung damage. The mechanisms accounting for these differences are presently unknown. Conditional TREK-1-deficient mice should give us insights into the molecular mechanisms responsible for the observed lung damage and help us resolve the discrepancies between in vitro and in vivo models. Once TREK-1 signaling pathways are delineated, the development of TREK-1-activating or -inhibiting agents may steer us toward new therapeutic approaches in ALI/ARDS. As with all known ion-channel modulators, drug-specificity issues will likely constitute future challenges, especially because K₂P channels are quite ubiquitously expressed in the body tissues.

The Lung Epithelium: NRG-1-HER2/3 Signaling in Epithelial Barrier Regulation

**HER family signaling: background.** Receptor tyrosine kinases, such as members of the human epidermal growth factor receptor (HER) family, and their ligands serve as important signaling molecules that regulate the epithelial response to extracellular signals and environmental stimuli. HER family signaling has been shown to regulate epithelial migration, growth, and differentiation as well as epithelial barrier function in numerous tissue beds including skin (184), intestine (38), cornea (123, 211, 215), and the lung (30). Identification of mutations of HER family genes as pathogenic in certain epithelial cancers (85, 180) has led to the successful deployment of targeted tyrosine kinase inhibitors (TKIs) aimed at inhibiting these receptors, particularly in breast and lung cancer (24, 105). However, recent data demonstrate that these receptors regulate pulmonary epithelial barrier function in nontransformed pulmonary epithelia, particularly in the context of inflammatory lung injury (46, 47).

The HER family is a complex signaling network consisting of four transmembrane receptors, HER1 or the EGFR, HER2, HER3, and HER4 and ten known ligands. The HER family is also referred to as the ErbB family after it was discovered that the avian erytheroblastosis virus gene encoded a mutant form of EGFR/HER1 (64, 65, 138). Members of the HER family are expressed on the basolateral surface of airway and AT I and II epithelial cells. The four HER receptors all share a similar structure including an extracellular ligand-binding domain, a
single transmembrane domain, and a cytoplasmic tyrosine kinase domain (209). HER receptors exist in quiescent cells as single “closed” monomers. Ligand binding induces a conformational change to an “open” configuration, allowing for homo- or heterodimerization, leading to phosphorylation of intracellular tyrosine residues and activation of the kinase domain. This allows for docking of cytoplasmic proteins that facilitate initiation of downstream signaling. Certain structural distinctions influence how specific monomers interact to form signaling competent dimer pairs. HER2 has no ligand yet, under basal conditions, exists in a receptive open confirmation, making it the preferred binding partner of other HER receptors. In contrast, HER3 has at least four different ligands yet does not have a functioning kinase and partners with other receptors to influence signaling. Although it does not directly phosphorylate other proteins, HER3 interacts with other intracellular proteins and signaling pathways such as the phosphatidylinositol 3 kinase pathway (103). Additionally, in cancer cells, HER3-dependent signaling is a resistance mechanism against HER2-targeted TKI therapy (52). Broadly, ligands are organized depending on which receptors they bind and activate. EGF, TGF-α, and amphiregulin bind EGFR/HER1 exclusively, whereas β-cellulin, heparin-binding EGF (HB-EGF) and epiregulin can bind EGFR/HER1 or HER4. The final group of ligands includes the neuregulins (NRG-1–4). The four NRG proteins all share a similar EGF-like domain, which is sufficient for receptor activation yet differs in tissue distribution structurally, which can influence signaling, NRG-1 being the isof orm most commonly studied.

HER family signaling is a complex, multilayered network influenced by factors such as dimerization partner, cell type (e.g., cancer vs. untransformed), and ligand. (148). For example, activated EGFR phosphorylates cCbl and Grb2 but only when activated in partnership with HER2, as opposed to other HER family members (58, 129). Additionally, whereas several ligands can bind and activate different HER receptors, each ligand elicits specific intracellular responses depending on cell type (e.g., transformed vs. nontransformed) and the availability of other HER receptors. In HER2-deficient cells, NRG-1 can induce EGFR/HER3 dimerization and activation, but when HER2 is available, NRG-1 preferentially induces HER2/3 heterodimers, eliciting specific intracellular signaling patterns (46, 58).

Accumulating data suggest that the HER family is important in regulating epithelial barrier function in the lung. Activation of EGFR with TGF-α leads to epithelial proliferation, spreading, and motility (99, 153) (86) and enhances wound closure in cultured AECs (86). Additionally, epithelial injury leads to shedding of HER family ligands, and wound closure is delayed after exposure to an EGFR inhibitor. Similarly, NRG-1 shedding and HER2 activation are also observed in a scratch model of wound closure, and blockade of HER2 impedes healing following wounding (200). Notably, exogenous NRG-1 increases epithelial permeability similar to a monolayer of epithelial cells (46). These data suggest that HER activation can enhance, improve, or worsen epithelial barrier function, possibly depending on the continuity of the barrier at the time of receptor activation.

**NRG-1-HER2/3: regulation of the pulmonary epithelial barrier.** Recent evidence has identified the NRG-1-HER2/3 system as activated in and as a critical modulator of pulmonary epithelial barrier function in ALI and ARDS. ALI and ARDS are syndromes marked by a hyperinflammatory state with an increase in cytokines such as IL-6 and IL-8 that are felt to participate in disease pathogenesis. In particular, the cytokine IL-1β has been implicated as a central mediator of ALI, both in animal models and humans (53, 71, 114, 132, 133, 140, 174). IL-1β is increased in the lavage of patients with ARDS, and exogenous IL-1β leads to increased epithelial permeability in an epithelial monolayer as well as in animal models (89). Therapeutically, strategies to block IL-1β signaling are protective in bleomycin and ventilator-induced models of lung injury (53, 132) (49). Epithelial NRG-1-HER2 signaling has been recently identified as activated by IL-1β and central in the pathogenesis of epithelial injury and permeability induced by IL-1β. In cultured airway and alveolar cells, IL-1β-mediated increases in epithelial permeability are HER2 dependent (46). IL-1β induces shedding of NRG-1 and HER2 activation without evidence of EGFR activation. The protease ADAM17 has been identified as the sheddase responsible for NRG-1 shedding. Pharmacological (inhibitors) or genetic (siRNA) strategies to inhibit ADAM17, NRG-1, or HER2 significantly attenuate IL-1β-mediated changes in epithelial barrier function. Hence, an overall pathway of IL-1β-ADAM17-NRG-1-HER2/3 leading to increased epithelial permeability has emerged as a new important regulator of epithelial barrier function in ALI.

NRG-1-HER2/3 signaling also participates in animal models of lung injury. Bleomycin-induced ALI is associated with both increased BAL NRG-1, indicative of surface shedding as well as pulmonary HER2 activation. Pharmacological ADAM17 inhibition attenuates NRG-1 clevage, pulmonary HER2 phosphorylation, and indices of injury in mice exposed to bleomycin. Bleomycin serves as a model of both acute and chronic fibrotic lung injury, and targeting HER2 is protective in the fibrotic phase as well as the acute phase of lung injury following bleomycin. Use of an inhibitor that prevents HER2 dimerization with HER3 prevented both HER2/3 activation as well as fibrosis at 21 days after bleomycin exposure (44). Similarly, transgenic mice lacking pulmonary epithelial HER3 are protected from bleomycin-induced fibrosis (125). Although in these studies indices of acute injury were not specifically studied, there was suggestion of protection in alveolar leak early following bleomycin. Mechanistically, alterations in alveolar epithelial permeability have been linked to a profibrotic response in the lung (2, 3, 15, 178), possibly providing a link between early acute injury marked by compromised alveolar epithelial barrier function and delayed fibrotic injury.

Proteases are critical participants in the HER family signaling, as they translate external signals into HER receptor activation through shedding of surface ligands. Numerous agonists that induce epithelia barrier dysfunction do so via protease-mediated HER ligand shedding, leading to receptor activation. Examples of this include IL-1β, mechanical stretch, and hypertonic stress. In particular, ADAM17 has been identified as the sheddase responsible for cleaving numerous HER family ligands and has been linked to disease marked by inflammation and increased permeability (21, 27, 29, 55, 211). In vitro, blocking ADAM17 reduces IL-1β-induced epithelial permeability through blocking NRG-1 shedding and subsequent HER2 activation. In vivo, intratracheal instillation of the ADAM17 inhibitor attenuated bleomycin-induced ALI, including protein leak. This was associated with decreased NRG-1 lavage levels and HER2 activation. Whereas ADAM17 KO mice display...
embryonic lethality, mice deficient in the endogenous inhibitor of ADAM17, tissue inhibitor of metalloprotease-3, have delayed resolution of lung injury following bleomycin administration, supporting the importance of this sheddase in ALI, although the role of HER ligands and receptors was not specifically investigated.

Perhaps the most compelling data indicating that the HER family participates in epithelial barrier disruption in the lung is that patients with ARDS have evidence of active ligand shedding in the lung. TGF-α is elevated in lavage from patients, suggesting pathway activation. Similarly, increased NRG-1 lavage levels have been detected from patients with ARDS. As a biomarker, NRG-1 levels were significantly correlated with inflammation as measured by inflammatory cytokines. Notably, NRG-1 elevations were associated with worse outcomes as measured by ventilator-free days. Plasma NRG-1 is also increased in patients with ARDS, suggesting that the source of NRG-1 leading to HER2 activation could be extrapulmonary.

**NRG-1-HER2/3 signaling: mechanisms regulating epithelial cell-cell adhesion and permeability.** Knowledge regarding the mechanisms by which HER2 and the other HER receptors influence epithelial permeability is incomplete, but several pathways have been outlined. HER proteins modulate cell-cell adhesion proteins of the tight junction and the adherens junction. EGFR alters claudin expression and spatial rearrangement of claudins and ZO-1 (28, 189). HER2 has also been linked to the tight junction proteins ZO-1 and occludin (54, 56), as well as integrin-β4 in regulating cell-cell adhesion (66). Recent data suggest that the adherens junction protein β-catenin is a required intermediary influencing HER2-mediated barrier function in the lung. Phosphorylation at tyrosine Y-654 of β-catenin has been linked to altered barrier function. NRG-1-mediated HER2 activation leads to β-catenin Y-654 phosphorylation as well as disruption of β-catenin-E-cadherin interaction at the cell junction in pulmonary epithelial cells (48). This is associated with impaired E-cadherin-mediated cell-cell adhesion. Finally, loss of β-catenin via shRNA knockdown results in a significant attenuation in NRG-1-HER2/3-induced barrier dysfunction. Together, these data suggest that NRG-1-HER3-HER2 activation leads to β-catenin tyrosine phosphorylation, disruption of adherens junction, and alteration of the pulmonary epithelial barrier (Fig. 3).

Additionally, processes inherent in barrier functions such as proliferation and cell migration suggest other pathways by which HER proteins might influence the epithelial barrier. The GTPases Rho and Rac have both been linked to HER family signaling. In a breast cancer epithelial cell line, HER2-dependent Rac activation was required for NRG-1-mediated cell migration (208). Rac activation following NRG-1 is likely via HER2-dependent P-Rex1 activation, as downregulation of P-Rex1 inhibited Rac activation and cell migration following NRG-1 (121). Similarly, EGFR activation leads to Rac activation and Rac-dependent cell migration (34). During epithelial wound closure, Rho activation follows EGFR activation, and, in a corneal epithelial wound model, Rho inhibition decreased both cell migration and proliferation, resulting in delayed wound closure (210).

Proteins involved in formation of tight junctions and adherens junctions are also mobilized after HER family activation. EGFR activation alters expression levels of claudins and the cellular localization of claudins and ZO-1 (28, 189). NRG-1-HER2 mediated changes in epithelial permeability are also
associated with alterations in cellular localization of the tight junction proteins ZO-1 and occludin (54, 56). Integrin-β4, an adhesion molecule that regulates cell-cell junctions (66) and epithelial proliferation (26), amplifies HER2 activation in epithelial cells to activate STAT and disrupt cell-cell-adhesion (60). Previous reports have suggested a structural and functional link between the adherens junction protein β-catenin and HER2. Structurally, HER2 colocalizes to the basolateral plasma membrane via binding to the PDZ domain of erbin (20). Erbin has been identified as a binding partner for the p120 catenin protein p0071 as well as direct binding partner for β-catenin (59, 147). In gastric and breast cancer epithelial cells, TGF-α induces EGFR-dependent HER2 activation, β-catenin phosphorylation, and β-catenin-HER2 association although the role of HER2 in β-catenin phosphorylation was not specifically studied (83, 128, 160, 170). Functionally, HER2 and β-catenin have both been linked to breast cancer pathogenesis. In murine breast cancer models utilizing a constitutively active HER2, β-catenin signaling was activated and required for tumor initiation and metastasis (155). Blockade of HER2 using trastuzumab blocked both HER2 activation and β-catenin phosphorylation, as well as β-catenin-dependent transcription of surviving breast cancer cells (214).

Conclusion

The airway epithelium is a physical and immunological barrier that sits at the interface between the lumen and the lung parenchyma and is the initial site of contact for all inspired substances. It is constantly exposed to both physiological and pathological stimuli and dynamically regulates its barrier. This dynamic regulation occurs via changes in cell-cell adhesion attributable to altered cytoskeletal and junctional protein arrangement and expression, as well as changes in transcellular and paracellular ion permeability in response to these stimuli to help maintain homeostasis. The ion channels on the apical and basolateral cell surfaces and the junctional complexes, which directly connect adjacent epithelial cells, play a key role in controlling the movement of substances from the lumen into the subepithelial space both via paracellular and transcellular transport. The junctions also separate the apical and basolateral cell surface, which maintains the specialized function of each cell surface, controls the interaction between various cytokines, growth factors, and their receptors, and modulates the extent and location of inflammatory response to stimuli. Exposure to noxious stimuli such as mechanical stretch/truma, HO, infection, cigarette smoke, or particulate matter damages the epithelial barrier, resulting in a loss of compartmentalization and a hyperproliferative, inflammatory state that is seen in many pulmonary diseases.

The junction proteins and ion channels interact to maintain lung fluid balance. Initial studies indicate that changes in the levels of the tight junction claudins can alter fluid balance. However, further work needs to be done to characterize the compensatory shifts in junction proteins that occur in response to physiological and pathological stimuli to maintain tissue homeostasis.

As outlined earlier, stretch activated ion channels play a role in epithelial barrier function. TREK-1 has been linked to the pathogenesis of ALI/ARDS; however, there are conflicting results in in vitro and in vivo models of ALI/ARDS. Future studies delineating the role of TREK-1 in ALI/ARDS could potentially lead to the development of new targeted therapies for this disease. The ENaC complex is another ion channel shown to be important in lung fluid balance, but better defining the role of the individual subunits in fluid balance as well as identifying other pathways that contribute to the trafficking of this complex will be important in further understanding the pathogenesis of diseases such as ARDS.

Although it is hypothesized that changes in the physical barrier in response to chronic stimulation by both physiological and pathological stimuli precipitate the immunological changes that lead to diseases such as COPD, ALI, and ARDs, the sequence and time course of these changes are yet to be determined. As discussed in this review, the epithelium dynamically regulates its response to a variety of stimuli to promote repair and regeneration; however, we do not understand mechanisms of dysregulation of this dynamic repair process. Future studies should further explore the link between altered transcellular and paracellular transport and the activation of the inflammatory cascades that result in chronic epithelial barrier dysfunction and injury. In addition, much attention has been devoted to pathways that mediate lung inflammation and injury; however, pathways that protect against lung injury are not well studied, and therefore few have been detailed in the literature. It is critical to note that Islam et al. (77) demonstrated that both epithelial injury and the downstream consequences of disease can be reversed by targeting the epithelium, which was done using mitochondrial transfer (77). However, this raises a future directive to, not only identify pathways involved in damage, but also investigate protective mechanisms. Recently, the apelin-APJ pathway has been shown to be protective against ARDS/ALI, but more work needs to be done to identify other pathways that protect against lung inflammation and injury (42). Our future directive is not to simply define and dissect disrupted molecular pathways in the epithelium but target and manipulate protective mechanisms to alter the course of disease. In conditions such as COPD, where there is a long lead time before the onset of disease, there is ample opportunity to prevent the development by manipulating epithelial targets. In conditions such as ARDS, we need to fine-tune mechanisms to target the epithelium to ameliorate the severity of disease and amplify repair and resolution.

As discussed earlier in this review, the maintenance of ion and fluid homeostasis in the lung is complex and requires the dynamic interaction between numerous ion channels and transporter proteins. Studies have started to explore the connections between paracellular and transcellular transport in maintaining fluid balance and ion homeostasis. However, our knowledge regarding this subject is still in its infancy. We have already identified certain transporters, such as aquaporin 5, which have roles in dictating both transcellular water transport and paracellular permeability as outlined in The Lung Epithelium: Insights into the Role of Regulation of Paracellular Permeability in Epithelial Signaling. Further defining the contribution of these transporters to the pathogenesis of different pulmonary diseases could potentially lead to the development of novel therapeutics. Indeed, Ivcavator, a drug approved to treat patients with cystic fibrosis who have specific mutations in the CFTR protein, is one such example, which could have reaches into noncystic fibrosis lung diseases as well.

In summary, the airway epithelium is a dynamic barrier that constantly responds to luminal stimuli and coordinates its response to maintain homeostasis in the lung. Breakdown in this coordinated response can lead to a myriad of lung diseases.
DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

K.B., J.H.F., and V.K.S. prepared figures; K.B., J.A.F., A.S., J.H.F., and V.K.S. drafted manuscript; V.K.S. edited and revised manuscript; V.K.S. approved final version of manuscript.

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NEW PLAYERS MODULATING THE EPITHELIAL BARRIER


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