



Inflammatory Responses Regulating Alveolar Ion Transport during Pulmonary Infections

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The respiratory epithelium is lined by a tightly balanced fluid layer that allows normal O₂ and CO₂ exchange and maintains surface tension and host defense. To maintain alveolar fluid homeostasis, both the integrity of the alveolar–capillary barrier and the expression of epithelial ion channels and pumps are necessary to establish a vectorial ion gradient. However, during pulmonary infection, auto- and/or paracrine-acting mediators induce pathophysiological changes of the alveolar–capillary barrier, altered expression of epithelial Na,K-ATPase and of epithelial ion channels including epithelial sodium channel and cystic fibrosis membrane conductance regulator, leading to the accumulation of edema and impaired alveolar fluid clearance. These mediators include classical pro-inflammatory cytokines such as TGF-β, TNF-α, interferons, or IL-1β that are released upon bacterial challenge with *Streptococcus pneumoniae*, *Klebsiella pneumoniae*, or *Mycoplasma pneumoniae* as well as in viral infection with influenza A virus, pathogenic coronaviruses, or respiratory syncytial virus. Moreover, the pro-apoptotic mediator TNF-related apoptosis-inducing ligand, extracellular nucleotides, or reactive oxygen species impair epithelial ion channel expression and function. Interestingly, during bacterial infection, alterations of ion transport function may serve as an additional feedback loop on the respiratory inflammatory profile, further aggravating disease progression. These changes lead to edema formation and impair edema clearance which results in suboptimal gas exchange causing hypoxemia and hypercapnia. Recent preclinical studies suggest that modulation of the alveolar–capillary fluid homeostasis could represent novel therapeutic approaches to improve outcomes in infection-induced lung injury.

Keywords: ion channel, ion pumps, edema, cytokines, Na-K-ATPase, cystic fibrosis membrane conductance regulator, epithelial sodium channel, lung injury

Abbreviations: AFC, alveolar fluid clearance; ALF, alveolar lining fluid; ALI, acute lung injury; AMPK, AMP-kinase; AQP, aquaporin; ARDS, acute respiratory distress syndrome; ASL, airway surface liquid; CaCC, Ca²⁺-activated ion channels; cAMP, cyclic AMP; CFTR, cystic fibrosis membrane conductance regulator; CNG, cyclic nucleotide-gated cation channel; ENaC, epithelial sodium channel; IAV, influenza A virus; ICAM, intercellular adhesion molecule-1; IFN, interferons; IL, interleukin; L-NMMA, N(omega)-monomethyl-L-arginine; LPS, lipopolysaccharide; MERS-CoV, middle east respiratory syndrome coronavirus; mRNA, messenger RNA; NETs, neutrophil extracellular traps; NKCC, Na⁺/K⁺/2Cl⁻ cotransporters; NO, nitric oxide; PECAM, platelet endothelial cell adhesion molecule-1; RONS, reactive oxygen and nitrogen species; ROS, reactive oxygen species; RSV, respiratory syncytial virus; SARS-CoV, severe acute respiratory syndrome coronavirus; TGF-β, transforming growth factor beta; TNFR1, TNF receptor 1; TNF-α, tumor necrosis factor alpha; TRAIL, TNF-related apoptosis-inducing ligand; VCAM, vascular adhesion molecule-1; β2AR, beta-2 adrenergic receptor.

INTRODUCTION

The major task of the respiratory tract is the exchange between inhaled atmospheric oxygen and carbon dioxide carried by the bloodstream, which is ensured by a thin but large surface area formed by type I and type II alveolar epithelial cells. Both the upper and the lower respiratory epithelia are lined by a thin (0.2 μM) aqueous layer (1), referred to as airway surface liquid (ASL) and alveolar lining fluid (AFL), respectively. This fluidic component serves—in concerted action with surfactant, mucus, and ciliary beat—to reduce alveolar surface tension and prevent atelectasis as well as to defend against invading pathogens. To maintain the composition of the ASL and AFL and to prevent alveolar flooding, lung fluid homeostasis is tightly controlled by the expression and activity of ion channels and pumps. These channels and pumps establish an osmotic gradient between airspace and interstitium, driving paracellular or aquaporin- (AQP3, 4, and 5) (2) mediated fluid movement across the respiratory epithelium. Among these, the apical amiloride-sensitive epithelial sodium channel (ENaC) and the amiloride-insensitive cyclic nucleotide-gated cation channel (CNG) acting together with the basolaterally located Na₂K-ATPase (NKA) promote transcellular sodium transport (3), which is accompanied in the alveolar epithelium by chloride uptake from the apical cystic fibrosis membrane conductance regulator (CFTR) (4). However, in the airway, CFTR promotes chloride secretion to regulate mucus density (5). In addition, Ca²⁺-activated ion channels (CaCC) promote apical chloride secretion, further supported by basolateral chloride uptake *via* Na⁺/K⁺/2Cl⁻ cotransporters (NKCC) (6) as well as potassium ion channels such as Kv7.1, contributing to cellular membrane potential and buildup of an electrochemical gradient necessary for apical chloride secretion (7). Additional factors influencing fluid homeostasis are epithelial (im)permeability established by tight junction proteins as well as endothelial integrity limiting the extravasation of fluid from the blood vessels driven by changes in the capillary hydrostatic pressure (8, 9).

Pulmonary infections commonly disturb ion and thus fluid homeostasis, resulting in abnormal changes of ASL, AFL, and alveolar edema formation. Both viral and bacterial pathogens are common causative agents for acute lung injury (ALI) and the acute respiratory distress syndrome (ARDS), which are characterized by a widespread inflammation within the lungs, extensive flooding of the alveolar airspace with protein-rich exudate fluid and impaired gas exchange leading to respiratory failure and resulting in mortality rates of 40–58% (10, 11). Additionally, sepsis resulting from primary infections at other sites is often complicated by the development of severe lung injury during the onset of bacteremia, resulting in lung failure and accounting for as many as half of all cases of ARDS (12). Although some of the pathogen-derived effects on ion transport during lung injury have been reported to be caused directly by the pathogen–host cell interaction (13), accumulating evidence suggests that auto- and paracrine mediators of local and/or systemic inflammatory responses mounted upon pathogen recognition and replication induce—among other pathophysiological changes—impaired ion transport and alveolar fluid clearance (AFC), resulting in edema formation and persistence. Importantly, mortality in ARDS

patients has repeatedly been found to correlate with persistence of alveolar edema (11, 14).

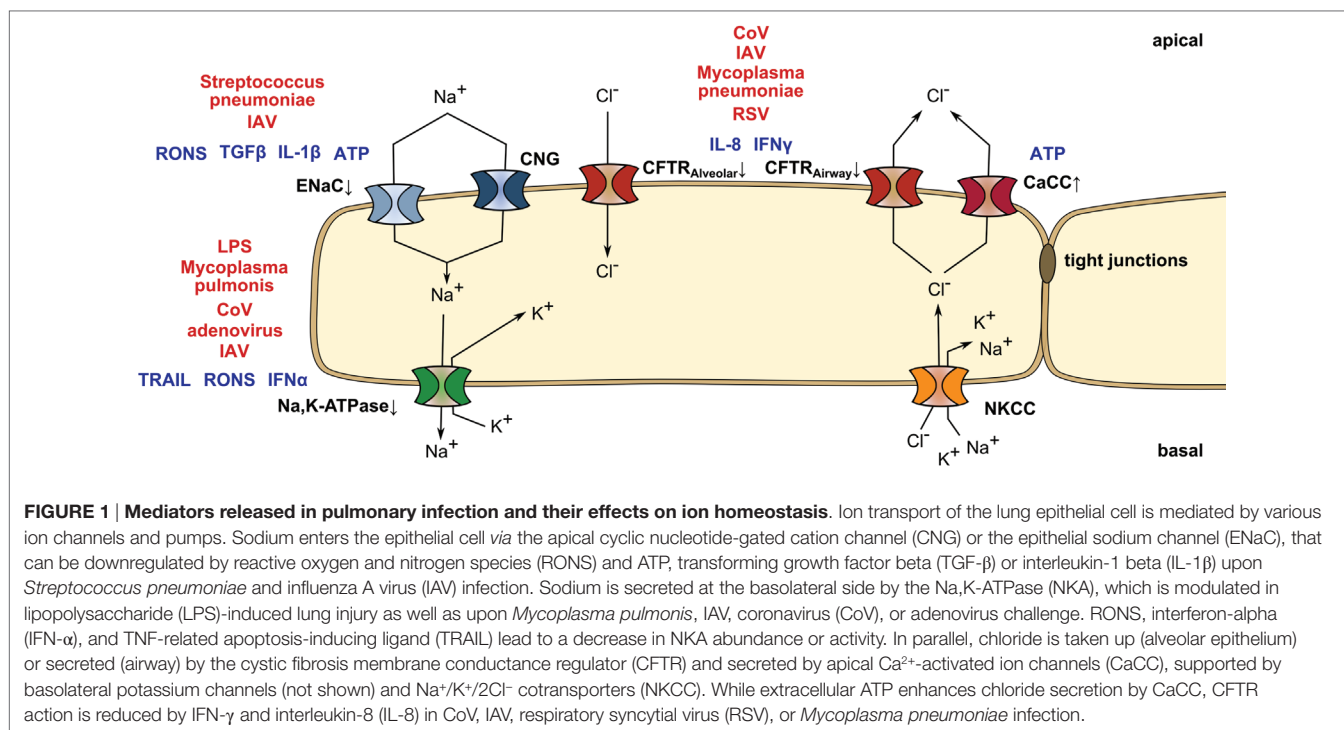
In this review, we will highlight advances in the understanding of how inflammatory responses in pulmonary infection affect ion transport, including common patterns and unique pathways activated by different respiratory pathogens, and how these mechanisms might be modulated to improve the outcomes of ARDS patients.

MEDIATORS MODULATING ION AND FLUID HOMEOSTASIS

There are numerous reports showing that pulmonary infection leads to loss of barrier integrity and edema accumulation as well as the role of distinct mediators on impairing ion channel or transporter function on the alveolar, bronchial, and gut epithelia. However, there have been few studies showing how infectious agents modulate soluble signaling molecules that affect ion and fluid homeostasis. Several reports from the last decade have reestablished an important role for soluble, inflammatory mediators in the progression of ARDS. For example, Lee et al. demonstrated that exposure of human ATII cells to pulmonary edema fluid derived from ARDS patients alone was sufficient to downregulate the ion channels and pumps involved in AFC, including ENaC, the NKA, and CFTR (15). Concomitantly, it was established that viral or bacterial lung infections lead to edema accumulation and impair clearance *via* the induction of paracrine factors. For example, influenza A virus (IAV) has been shown to increase apical potassium secretion by upregulation of the apical potassium channel KCNN4 by a paracrine signaling event, thus disturbing the osmotic gradient necessary for edema clearance (16). Similarly, *Pseudomonas aeruginosa* evokes a strong inflammatory response and lung edema accumulation related with the modulation of ENaC subunit expression (17, 18). In the next paragraphs, we will provide an overview on interconnections of mediators released in pulmonary infection and their effects on ion and fluid homeostasis (Figure 1).

Interferon

Once cells detect pathogens by their specific and specialized pattern recognition receptors, they produce interferons (IFN), which can be detected—if not actively suppressed by a given pathogen—in most pulmonary infection scenarios. Effects of IFN on fluid homeostasis seem to be mostly limited to gamma IFN (IFN- γ), which have been attributed a modulatory role in both innate and adaptive immunity (19, 20). IFN- γ has been reported to decrease sodium transport at levels as low as 10 U/ml (21). Moreover, IFN- γ can also directly decrease chloride currents along the bronchial epithelium by downregulating CFTR due to a posttranscriptional modulation of CFTR messenger RNA (mRNA) stability and thus half-life (21–23). In contrast, both class I IFN, IFN- α , and IFN- β that are usually implicated in mounting a direct cellular pathogen-restrictive response do not modulate CFTR mRNA or protein abundance (22). IFN- α appears to negatively impact NKA cell membrane protein abundance during IAV infection *via* activating the metabolic sensor AMP-kinase (AMPK) (24). However, to date,



there is no data supporting whether this effect of IFN-α on ion transport is a generalized response during pulmonary infections.

Tumor Necrosis Factor Alpha (TNF-α)

Tumor necrosis factor alpha is a classical cytokine produced upon local or systemic inflammation, regulating differential processes such as proliferation and differentiation of immune cells as well as cell death (25–27). After initial conflicting studies, it has by now become clear that it plays a dichotomic role in lung fluid reabsorption (28). On one hand, TNF-α ligation to its receptor TNF receptor 1 (TNFR1, also named CD120a or p55) inhibits ENaC activity both *in vitro* and *in vivo* via a PKC-dependent mechanism (29). On the other hand, a distinct lectin-like domain of TNF different from the receptor-binding domain, which can be mimicked by the 17-amino acid circular TIP peptide (30), has been reported to increase edema reabsorption in rat bacterial pneumonia (31). Application of the TIP peptide has been demonstrated to elevate ENaC expression and open probability (32) resulting in enhanced AFC in *P. aeruginosa*-treated rats *in vivo* (31) and has furthermore been reported to increase NKA activity (33). In addition to its direct effects on ion channels and pumps of the alveolar epithelium, the TNF-α/TNFR1 interaction also modulates the integrity of the alveolar barrier, as it increases endothelial expression of chemoattractants and adhesion molecules including the interleukin-8 (IL-8; formerly called neutrophil chemotactic factor)/IL-8-receptor 2 axis, the intercellular adhesion molecule-1, platelet endothelial cell adhesion molecule-1, and vascular adhesion molecule-1, and thus promotes excessive recruitment of mononuclear phagocytes and neutrophils during lung inflammation (30, 34, 35). Importantly, besides cellular transmigration itself,

neutrophil-derived proteases and neutrophil extracellular traps are central drivers of both endothelial and epithelial injury (36).

Interleukin-1 Beta (IL-1β)

Interleukin-1 beta is one of the most commonly found cytokines in pulmonary edema and bronchoalveolar lavage fluids in experimental and human ARDS (37, 38) and is, for example, induced during *Klebsiella pneumoniae* bacterial pneumonia (39–41). It is mainly produced by macrophages and, similarly to TNF-α, has a major impact on cell proliferation, differentiation, and cell death. In pulmonary inflammation, IL-1β increases lung barrier permeability in *in vitro* and *in vivo* models of ARDS (41, 42) and may contribute to alveolar edema in lung injury models by impairing fluid reabsorption from the lungs. This can in part be attributed to decreased sodium absorption due to a decrease in αENaC expression and trafficking to the apical membrane of ATEC cells (43). In addition, IL-1β in *Streptococcus pneumoniae* infection (44)—and also TNF-α and IFN-γ (45)—can influence ion transport processes *via* activation of the pro-coagulant factors (46). Thrombin in particular has been demonstrated to impair AFC by increasing the PKC-ζ-dependent endocytosis of the alveolar NKA (47).

Interleukin-8

Interleukin-8 is a chemotactic factor that correlates with neutrophil accumulation in distal airspaces of patients with ARDS and is a predictor of mortality (48–50). IL-8 is secreted by bronchial epithelial cells and can be induced by *Mycoplasma pneumoniae* antigen or live *M. pneumoniae* (51) as well as by severe acute respiratory syndrome coronavirus spike protein or respiratory syncytial virus infection (52, 53). The rate of AFC is impaired by high levels of IL-8 and is significantly lower in patients who have

a pulmonary edema fluid concentration of IL-8 above 4,000 pg/ml (54). Mechanistically, IL-8 inhibits beta-2 adrenergic receptor (β 2AR) agonist-stimulated fluid transport across rat and human alveolar epithelia. This inhibition is mediated by a PI3K-dependent desensitization and downregulation of the β 2AR from the cell membrane associated with an inhibition of cyclic AMP generation normally observed in response to β 2AR agonist stimulation (54).

Transforming Growth Factor Beta (TGF- β)

The cytokine TGF- β is a critical factor for the development of ARDS. Besides its established role in dampening inflammatory responses (55), e.g., by driving macrophages toward an anti-inflammatory phenotype (56), it increases alveolar epithelial permeability to promote edema formation upon lipopolysaccharide (LPS) stimulation (57). Furthermore, TGF- β has been shown to inhibit amiloride-sensitive sodium transport by an ERK1/2-dependent inhibition of the α ENaC subunit promoter activity, decreasing α ENaC mRNA and protein expression (58). In addition, Peters et al. (59) demonstrated that TGF- β leads to the subsequent activation of phospholipase D1, phosphatidylinositol-4-phosphate 5-kinase 1 α , and NADPH oxidase 4 (Nox4). Nox4 activation results in the production of reactive oxygen species (ROS) that in turn reduce cell surface stability of the $\alpha\beta$ ENaC complex and thus promote edema fluid accumulation. Moreover, TGF- β decreases NKA β 1 subunit expression, resulting in decreased NKA activity in lung epithelial cells (60, 61). In further support of a role for TGF- β in lung injury, TGF- β levels are increased in lung fluids from patients with ALI/ARDS (62) and in murine models of *Streptococcus pneumoniae* and IAV infection (63, 64). Of note, TGF- β has been proposed to further aggravate edema formation in IAV infection by increasing epithelial cell death, causing a disruption of epithelial barrier integrity (64). Moreover, it has been implicated in the upregulation of cellular adhesins which increase host susceptibility to bacterial co-infections (65) posing a major risk for increased viral pneumonia-associated morbidity and mortality during influenza epidemics (66).

TNF-Related Apoptosis-Inducing Ligand (TRAIL)

The principal role of TRAIL, highly released by lung macrophages upon viral infection, is to drive infected cells into apoptosis to limit pathogen spread. TRAIL has been reported to be produced especially during viral respiratory infections, including IAV-, adenovirus-, and paramyxovirus infection, and cell sensitivity to TRAIL-induced apoptosis is enhanced in infected cells by increased TRAIL-receptor expression (67, 68). However, this process also affects alveolar epithelial barrier integrity leading to edema accumulation (67, 69). Moreover, TRAIL signaling leads to NKA downregulation in IAV infection in non-infected neighboring alveolar epithelial cells mediated by AMPK (24). Accordingly, TRAIL signaling reduces AFC and promotes edema formation. In addition, TRAIL release upon IAV infection further favors bacterial superinfection with *S. pneumoniae*, aggravating lung injury (70).

Nucleotides

During acute infection, extracellular nucleotides often serve as danger signals involved in recognition and control of pathogens by promoting the recruitment of inflammatory cells, stimulating pro-inflammatory cytokines, and increasing the production of ROS or nitric oxide (NO) (71, 72). Extracellular ATP, which can be released from the airway epithelia and is produced by endothelial cells upon acute inflammation, binds to P2 purinergic receptors to promote a calcium signaling-dependent stimulation of CaCC and a decreased open probability of ENaC (73, 74). Moreover, extracellular adenosine, produced from ATP by hydrolysis via the ecto-5'-nucleotidase CD73, is increased in bronchoalveolar lavage fluid of IAV-infected mice, and genetic deletion of the A1-adenosine-receptor is protective (75). However, CD73 is only to a limited extent involved in the progression of lung injury and has no effect on pulmonary edema formation (76).

Reactive Oxygen and Nitrogen Species (RONS)

Reactive oxygen and nitrogen species have been shown to be involved in the development of epithelial injury in pathologic situations, including LPS-/sepsis-induced lung injury as well as viral pneumonia, in which RONS are produced in large quantities by alveolar phagocytes (77). Studies in rabbit and piglet lungs further elucidated that RONS affect AFC and edema persistence by inhibiting both the activity of ENaC and alveolar epithelial NKA (78, 79).

EFFECTS OF ION CHANGES ON CYTOKINE PRODUCTION

To add to the complexity of airway and alveolar fluid regulation, it has been suggested that not only ion channels, pumps, and transporters are modulated by signaling factors released upon pulmonary infection but also changes in ion transport influence the respiratory inflammatory response. For example, the transporter NKCC1—which plays a critical role in basolateral ion transport—can affect the severity of pneumonia and sepsis and consequently severity of lung injury, by regulating the ability of the alveolar-capillary barrier to modulate neutrophil infiltration into the air spaces of the lung (80). Lack of NKCC1 in a mouse model of pneumonia infection with *K. pneumoniae* or LPS resulted in increased numbers of neutrophils in the lavage fluid, decreased bacteremia, and importantly mortality. It has, therefore, been suggested that the activity of NKCC1 contributes to edema formation and decreased neutrophil migration into the lung air spaces, probably contributing to reduce bacterial killing and the subsequent development of severe sepsis (81–83). Similarly, mutations of CFTR can amplify lung inflammation by upregulating pro-inflammatory responses caused by an increase in cytokine production upon NF κ B activation in lung epithelial cells (84). Lack of functional neutrophilic CFTR in a model of LPS-induced lung inflammation contributes to inflammatory imbalance with NF κ B translocation and a reduction of anti-inflammatory cytokines such as IL-10, favoring the increase in lung vascular permeability (85). Also ion imbalances in

response to expression of viral ion channels or viroporins, has been recognized as potential pathogen recognition pathway that favors inflammasome activation and the release of IL-1 β , TNF, and IL-6, which might contribute to the limitation of virus spreading (86, 87).

THERAPEUTIC MODULATION OF THE ALVEOLAR–CAPILLARY FLUID BALANCE DURING PULMONARY INFECTION

As stated above, pulmonary infections—especially in severe cases—can lead to lung edema accumulation and impaired edema clearance. Lung edema results in impaired oxygenation and organ dysfunction which if not resolved leads to high mortality of patients with ARDS (11, 14). Current treatment options for infection-induced ARDS include antivirals and antibiotics. However, there is increased antibiotic resistance—reported for pathogens such as *K. pneumoniae*, *Escherichia coli*, *Staphylococcus aureus* and *P. aeruginosa* (82, 83, 88)—or lack of readily available treatment options for some acute emerging agents such as zoonotic influenza viruses or middle east respiratory syndrome coronavirus (89–91). Current approaches to treat ARDS patients include low tidal volume mechanical ventilation, positive end expiratory pressure, fluid management, and extracorporeal membrane oxygenation as measures to primarily improve oxygenation (92). Interestingly, lung-protective ventilation strategies have not only been reported to reduce mortality by 22% in patients with ARDS but also to diminish the number of neutrophils and the concentration of pro-inflammatory cytokines released in patient lavage fluids.

Novel approaches targeting host mediators known to promote lung edema formation and impair clearance such as studies on TIP peptide [see Tumor Necrosis Factor Alpha (TNF- α) above] administration in ARDS are being studied. Initial reports showed that AP301, a synthetic peptide mimicking TIP, induces ENaC activity in type II alveolar epithelial cells from dogs, pigs, and rats (93) and improves lung function in a porcine lung injury model (94). A subsequent phase II clinical trial with AP301 in ventilated ARDS patients resulted in improved AFC and oxygenation of these patients (95). Also, mesenchymal stem cells, which have been reported to improve epithelial barrier integrity in human

AEC II treated with a cytokine mix composed of a combination of IL-1 β , TNF α , and IFN γ (96), are currently tested for safety and efficacy in phase II trials (clinical trial identifiers NCT02097641, NCT01775774, NCT02112500). Studies on β 2-agonists, which had been previously shown to improve vectorial sodium transport and edema clearance (97, 98), did not improve ARDS outcomes (99, 100), possibly due to an enhanced inflammatory response driven by lung macrophages (101). Further treatment options targeting para- or autocrine signaling events affecting AFC in preclinical models include glucocorticoids that suppress inflammation and upregulate both NKA (102) and ENaC (103, 104), neutralizing antibodies directed against virus-specific release of macrophage TRAIL that improve NKA expression as well as AFC in IAV-infected mice (24) and nitric oxide synthase inhibitors aminoguanidine or N(omega)-monomethyl-L-arginine (L-NMMA) that protect against pulmonary edema in LPS-induced lung injury as well as in IAV infection (77, 105).

CONCLUSION

Pathogen-induced lung injury but also sepsis can lead to widespread respiratory inflammation that favors accumulation of lung edema leading to multiorgan dysfunction and poor outcomes. Recent advances in the development of novel treatment strategies targeting respiratory ion homeostasis show encouraging results, identifying them as promising candidates to improve AFC in ALI which could potentially improve the survival of patients with ARDS.

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CP, SH, JS, and EL have performed bibliographic research and drafted the manuscript.

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Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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