Review Article

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Crosstalk signaling between alveoli and capillaries

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Abstract

Crosstalk signaling between the closely juxtaposed epithelial and endothelial membranes of pulmonary alveoli establishes the lung's immune defense against inhaled and blood-borne pathogens. The crosstalk can occur in a forward direction, as from alveolus to capillary, or in a reverse direction, as from capillary to alveolus. The crosstalk direction likely depends on the site at which pathogens first initiate signaling. Thus, forward crosstalk may occur when inhaled pathogens encounter the alveolar epithelium, while reverse crosstalk may result from interactions of blood-borne pathogens with the endothelium. Here, we review the factors that regulate these two directions of signaling.

Keywords

alveolar, ARDS, acute respiratory distress syndromes, acute lung injury, capillaries, lung repair and regeneration

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A major function of the lung is to develop a defensive immune response to pathogens that might reach the alveolar gas-exchanging regions by inhalation or through blood-borne delivery. To develop the response, proinflammatory signals must pass between the alveolar gas and blood compartments across the alveolar–capillary barrier, which is formed by juxtaposed epithelial and endothelial membranes in the alveolar wall. However, the barrier, formed by tight and adherens junctions of the epithelial–endothelial double membrane, highly restricts liquid fluxes between the compartments. This restriction maintains, at a minimum, metabolically required liquid fluxes that if unfettered could lead to edema, impeding gas exchange. Hence, the question we consider here relates to how proinflammatory signals negotiate the flux-restrictive alveolar–capillary barrier to induce an effective immune defense.

In the alveolar wall, the close apposition of epithelium to endothelium, which may be separated by distances in the micron to submicron range,[1,2] strongly suggests that direct crosstalk exists between these membranes. We considered the possibility that because of their proximity, the membranes might interact through gap junctional communication (GJC). Hence, we carried out fluorescence-recovery-after-photobleaching (FRAP) studies by means of real-time fluorescence imaging (RFI) of mouse lungs. Although our reported studies revealed strong evidence for inter-epithelial and inter-endothelial GJCs,[3,4] by FRAP we failed to find evidence for epithelial–endothelial GJCs (unreported findings), indicating, to our knowledge, cross-compartmental GJC is not the basis of alveolar-capillary crosstalk.

It follows that paracrine signaling between epithelium and endothelium could account for the crosstalk. Thus, inhaled pathogens such as bacteria that encounter the alveolar epithelium are likely to activate epithelial–endothelial crosstalk in the forward direction, namely through signaling mechanisms that sequentially step through the apical and

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basolateral aspects first of the epithelial, then the endothelial membrane. On the other hand, a similar step-through signaling in the reverse direction could occur following primary activation of the endothelium. The final common pathway that leads to alveolar inflammation and injury involves neutrophil recruitment to the endothelium followed by neutrophil-induced barrier injury first to the endothelium, then to the epithelium as neutrophils migrate across the endothelium and develop direct epithelial–neutrophil interactions. Here we review the conditions under which forward and reverse crosstalk signaling have been addressed in the understanding of neutrophil-mediated alveolar injury.

Crosstalk from alveolus to capillary

Crosstalk signaling in the forward (alveolar–capillary) direction has been considered largely in the context of acute lung injury (ALI), which leads to the high-mortality acute respiratory distress syndrome (ARDS).[5] Characteristics of ALI, which typically follow lung infection by inhaled bacteria,[5,6] include neutrophil and platelet activation, coagulation in pulmonary vessels, and alveolar edema.[6] These responses usually follow activation of resident alveolar macrophages.[5] The major crosstalk mechanisms involved may be grouped as follows.

Alveolar cytokines

In ALI, airway pathogens cause release of macrophage-derived cytokines such as TNFα, which induce leukocyte recruitment from microvessels.[7,8] These events can be directly visualized by real-time fluorescence imaging (RFI) of rodent lungs.[1] As proof–of–principle of how cytokines released in the alveolar space might signal to the endothelium, RFI was carried out following alveolar microinjections of TNFα. These microinjections increased epithelial Ca^{2+}, following which there was increase of endothelial Ca^{2+}, which caused endothelial P-selectin expression. Inhibition of cytosolic phospholipase A2 blocked the TNFα-induced inflammatory response, implicating epithelial arachidonate as a crosstalk mediator.[9] Subsequent events leading to neutrophil recruitment are likely to involve endothelial cytokine release, as exemplified by the smoke inhalation model of ALI. Thus, intravenous delivery of anti-IL-8 antibody blocked smoke inhalation-induced ALI.[21] Since IL-8 causes neutrophil recruitment, these findings indicate that smoke inhalation resulted in endothelial IL-8 release, leading to lung recruitment of activated of neutrophils.

Epithelial effects of lung expansion

Another example of alveolar–capillary crosstalk was revealed through RFI studies of lung expansion.[10] Brief lung expansion increased cytosolic Ca^{2+} in both alveolar epithelia and microvascular endothelia, and NO production in endothelia. Prolonged lung expansion induced endothelial nitrotyrosine, indicating onset of nitrosative stress. Expansion-induced endothelial responses for cytosolic Ca^{2+} and NO were inhibited by alveolar infusion of a purinergic receptor antagonist, as well as in mice lacking the purinergic P2Y2, but not the P2Y1 receptor. Thus, these studies identify ATP as a paracrine mediator of alveolar–capillary crosstalk, in that the Ca^{2+}-dependent basolateral release of epithelial ATP induced P2Y2-mediated endothelial NO production.

Acid aspiration

Alveolar inflammation due to aspiration of concentrated hydrochloric acid (HCl) is a major cause of ALI.[5,6] Since HCl does not have a known receptor, the mechanisms underlying inflammation remain puzzling. Airway HCl instillation, which models the disease, results in pulmonary edema and inflammation, as indicated by an increase in extravascular lung water[11,12] and the accumulation of leukocytes in the bronchoalveolar lavage (BAL).[12] Airway acid instillation also increases endothelial P-selectin, vWf, and tissue factor expression. These responses are inhibited in the NOX2-deficient gp91phox$^{-/-}$ mouse, indicating that the crosstalk is reactive oxygen species (ROS)-dependent.[13]

In RFI studies, alveolar microinjection of HCl caused transient pore formation in alveolar epithelial cell membranes (Fig. 1).[11] Ca^{2+} entry through the pores induced Nox2-dependent epithelial H_2O_2 release. Consequently, H_2O_2, detected as increase of fluorescence of the dye DCF, increased in the neighboring microvascular endothelium. Alveolar macrophage depletion by clodronate pretreatment failed to block the endothelial H_2O_2 increase, ruling out a macrophage role in the response. These findings indicate that instillation of alveolar HCl causes a receptor-independent alveolar–capillary crosstalk in that mechanical damage to the epithelial cell membrane communicates injury signals to the endothelium by paracrine effects of H_2O_2.

Crosstalk from capillary to alveolus

Injury or infection in extra-pulmonary organs can cause ALI,[5,6] indicating blood-borne proinflammatory factors are capable of inducing injury to the alveolar–capillary barrier. However, it remains unclear as to how the lung endothelium conveys proinflammatory signals to the alveolar epithelium. RFI studies indicate that for some receptor-mediated mechanisms, the signaling occurs only in the forward (alveolar–capillary) direction. This is evident for TNFα and ATP, neither of which induces epithelial responses when given as capillary injections,[9,10] indicating that these proinflammatory factors do not support reverse (capillary–alveolar) crosstalk. Since direct evidence is lacking that small molecular intermediates secreted from the abluminal aspect of the endothelium subserve proinflammatory endothelial–epithelial interactions, it is likely that endothelium-activated neutrophils migrate to the epithelium

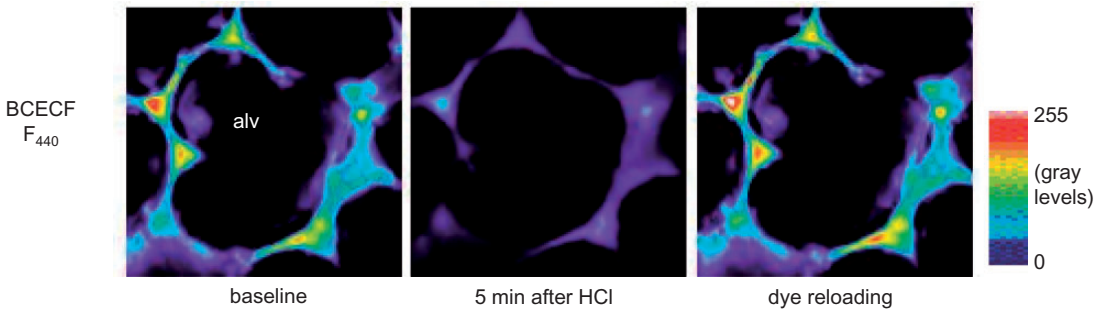

Fig. 1. Effect of HCl on alveolar epithelium. Pseudocolor images show cytosolic BCECF fluorescence at 440 nm (F_{440}) in the alveolar epithelium. Middle image was acquired 5 min after alveolar HCl infusion of pH 1.5. Right image was acquired after reloading the alveolus with BCECF 30 min after the HCl infusion. Alv, alveolar lumen; BCECF, 2-,7-Bis(2-carboxyethyl)-5-(and 6)-carboxyfluorescein. Reprinted from Westphalen et al.[11]

to evoke proinflammatory consequences. We consider conditions in which a primary effect on the endothelium results in alveolar injury.

Endothelial effects of lung expansion

High tidal volume mechanical ventilation (HTV), defined as tidal volumes $\geq$12 mL/kg, causes ALI.[14] HTV causes stretch-induced endothelial Ca^{2+} entry via TRPV4 channels,[15] leading to increased leukocytes in the bronchoalveolar lavage (BAL).[16,17] As determined in freshly isolated lung endothelial cells (FLEC), HTV increases endothelial tyrosine phosphorylation of the focal adhesion protein paxillin and paxillin-associated expression of the neutrophil adhesion receptor P-selectin.[18] Depletion of platelets and leukocytes decreased the induced P-selectin expression.[19] Further, HTV increased BAL content of albumin and neutrophils in chimeric mice that received IL-6-knockout hematopoietic cells, indicating that neutrophil-derived IL-6 was protective against HTV-induced ALI.[22]

HTV also induced cell-surface von Willebrand factor (vWF) expression.[20] Intravenous infusion of P-selectin antibody[19,20] or perfusion of lungs of P-selectin knockout mice with autologous blood[20] blocked the HTV-induced endothelial protein tyrosine phosphorylation and P-selectin expression. The inhibition failed in P-selectin-null lungs perfused with wild-type blood. These studies revealed an inflammation potentiating mechanism in which platelet P-selectin mediates vWF transfer to the endothelial cell surface.[20] Hence, a major effect of lung stretch is to induce endothelial vWF and P-selectin expression, leading to recruitment of inflammatory cells that migrate to cause epithelial injury.

Hypoxia

In ALI,[21,22] hypoxic pulmonary vasoconstriction (HPV) can cause ventilation-perfusion mismatch by redistributing pulmonary blood flow and exacerbating hypoxemia. The HPV may result from hypoxia-induced alveolar–capillary crosstalk.[23] Until recently, it was thought that arterioles are the site of hypoxia sensing. However, recent RFI evidence identifies the alveolar capillary endothelium as the site of hypoxia detection (Fig. 2). Thus, hypoxia depolarized the endothelium, possibly by inhibiting oxygen-sensitive voltage-gated K^+ channels, causing endothelial Ca^{2+} entry.[24] The Ca^{2+} increase propagated upstream to the adjoining arteriolar endothelium through connexin 40-containing gap junctions. Subsequently, activation of α_{1G}-type Ca^{2+} channels and of cytosolic phospholipase A_2 mediated release of epoxyeicosatrienoic acids. Together, these responses caused smooth muscle contraction, hence arteriolar vasoconstriction. Thus, HPV is an example of capillary–alveolar crosstalk in which an endothelial ion channel senses alveolar hypoxia, initiating arteriolar vasoconstriction.

Hypoxic RBC

Systemic hypoxia, a major hallmark of ALI, results from pulmonary edema and severe lung inflammation. Hypoxia itself is capable of causing lung inflammation in animal models[25–27] and humans,[28,29] predisposing to ALI. RFI studies in hypoxia-exposed lungs revealed an unusual example of intercellular crosstalk, namely that occurring between RBCs and the endothelium. Hypoxia increased microvascular ROS and cytosolic Ca^{2+}, leading to P-selectin-dependent leukocyte recruitment.[30] These responses were inhibited in the absence of erythrocytes in the perfusion solution. Inhibition of hemoglobin autoxidation with carbon monoxide or nitrite blocked hypoxia-induced responses, as did the infusion of catalase. Enhanced hypoxia responses occurred in BERK-trait mice, which are excessively susceptible to hemoglobin autoxidation. These data suggest that in lungs exposed to hypoxia, increased hemoglobin autoxidation causes superoxide production in erythrocytes. Superoxide dismutates to H_2O_2, which diffuses to microvascular endothelium initiating Ca^{2+}-dependent leukocyte recruitment. Accordingly, in lungs exposed to hypoxia (8% O_2) for 4 h, leukocyte adhesion receptor expression, ROS, and protein tyrosine phosphorylation increased in the endothelia, while leukocyte numbers increased in the BAL. Extracellular catalase and erythrocyte removal inhibited the hypoxia effects, mechanistically implicating erythrocyte-derived H_2O_2 in the

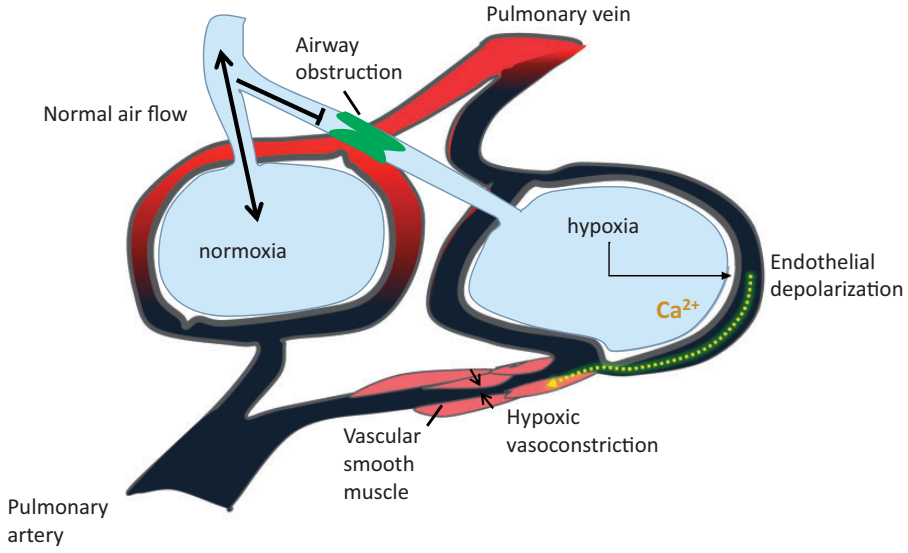

Fig. 2. Capillary mechanism of HPV. Mixed venous blood flows in through the pulmonary artery then branches to arterioles and then alveolar capillaries. The vascular smooth muscle ends at the arteriolar level. Capillaries surround air-filled alveoli. In the normally ventilated alveolus (left), the hypoxic blood (black) becomes oxygenated (red) and leaves the lung through the pulmonary veins. Airway obstruction causes alveolar hypoxia (right). The capillary endothelium is depolarized. The resulting increase of endothelial Ca^{2+} is communicated through endothelial gap junctions to smooth muscle surrounding upstream arterioles (yellow dotted line with arrow). Smooth muscle activation causes vasoconstriction, restricting blood flow in the under-ventilated region.

inflammatory response.[31] Thus in hypoxia-induced lung injury, H_2O_2 induced by hemoglobin autoxidation likely mediates RBC-endothelial crosstalk.

RBC transfusion

Transfusion of RBCs is a risk factor for ALI.[32–36] The morbidity risk appears to relate directly to RBC storage duration.[37,38] The blood group antigen Duffy,[39] advanced glycation end products (AGE),[40,41] and necroptosis mediators[42] have been implicated as possible mediators of RBC-induced lung injury. RBC expression of blood group antigen Duffy, a chemokine scavenger,[43] decreases with storage of human RBCs.[39] In a two-hit model of ALI in which vascular infusion of endotoxin was followed by transfusion of stored RBCs, lung microvascular permeability and BAL neutrophil counts increased.[39] Fresh RBCs had no effect. Transfusion of Duffy–knockout RBCs in wild-type mice worsened the injury, suggesting that Duffy antigen protects against RBC-induced ALI.[39]

Prolonged RBC storage associates with increased formation of AGE.[40] Human pulmonary microvascular endothelial cells (HMVEC) express the receptor for advanced glycation end products (RAGE). Stored RBCs increased ROS formation in HMVEC, an effect which was blocked by soluble RAGE and anti-RAGE antibody. Subsequently, RBC transfusion was shown to increase lung endothelial RAGE expression, as well as expression of damage-associated molecular patterns, high mobility group box 1 (HMGB1) and vascular cell adhesion molecule 1.[41] Transfusion of RBCs into RAGE knockout mice mitigated these proinflammatory effects. Taken together, these data suggest AGE–RAGE interactions may underlie RBC-mediated lung injury.

A role for endothelial necroptosis in RBC transfusion-associated ALI was recently reported. Following exposure to allogeneic RBCs, human lung endothelial cells in culture released HMGB1.[42] Cultured endothelial cells also underwent necroptosis, releasing the necroptosis mediator RIP3. Septic patients who received blood transfusions were noted to have higher plasma RIP3 levels, as were septic patients who died. In a two-hit animal model of ALI induced by RBC transfusion then LPS injection, transfusion of stored RBCs increased BAL cytokines. Administration of HMGB1 antibody blocked this effect. Although these findings implicate RBC-induced endothelial HMGB1 release in capillary–alveolar crosstalk in ALI, further studies are required to clarify underlying mechanisms.

Lung regeneration

Although, as we note above, definitive evidence is lacking that the endothelium secretes small-signaling intermediates from the abluminal aspect, several studies implicate endothelial proteins in reverse crosstalk. Thus, in a model of lung regeneration after pneumonectomy, endothelial-specific knockout of Vegfr1 and Fgfr1 impaired alveolarization,[44] suggesting an essential role for endothelia in the formation of alveolar epithelium. The authors mechanistically implicate matrix metalloprotease-(MMP)14 in the capillary–alveolar crosstalk, as intravascular replacement of MMP-14+ pulmonary capillary endothelial cells rescued

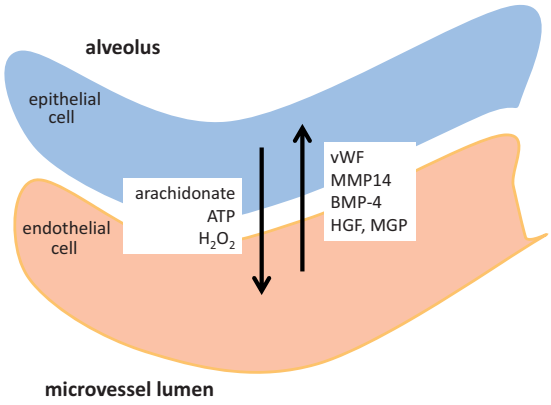

Fig. 3. Alveolar–capillary and capillary–alveolar crosstalk mediators. Epithelial cells line the alveolus and endothelial cells line the microvessels. In ALI, small molecules including ATP, arachidonate, and H_2O_2 have been implicated in alveolar–capillary crosstalk mechanisms. In ALI and in lung regeneration, protein mediators such as vWF, MMP14, BMP-4, HGF, and MGP have been implicated in reverse crosstalk mechanisms.
vWF: von Willebrand factor; MMP14: matrix metalloprotease 14; BMP-4: bone morphogenic protein 4; HGF: hepatic growth factor; MGP: matrix Gla protein.

post-pneumonectomy alveolarization in mice lacking Vegfr1/Fgfr1 specifically in the endothelium. These data suggest that capillary–alveolar crosstalk, mediated by MMP14-dependent activation of Vegfr2 and Fgfr1 in the endothelium, is required for alveolar regeneration.

Platelets have also been implicated as mediators of endothelial-driven alveolar regeneration in a pneumonectomy model. Using a combination of infusion of knockout platelets and endothelial-specific deletion of stromal-cell-derived factor 1 (SDF-1) receptors, the authors showed that platelets activate SDF-1 receptors on lung endothelial cells, which then causes a MMP14-induced alveolar epithelial cell expansion. Of note, endothelial-specific deletion of *Mmp14* inhibited epithelial, but not endothelial expansion, suggesting crosstalk from endothelium to epithelium in lung regeneration.[45]

Capillary–alveolar crosstalk in bronchoalveolar stem cell (BASC) differentiation was also recently demonstrated. BASCs located in the bronchoalveolar duct junction can differentiate into bronchiolar or alveolar cells. Using clonal 3D co-cultures of BASCs and endothelial cells, the authors identified a bone morphogenetic protein-4 (BMP4)-activated nuclear factor of activated T cell (NFAT) c1-thrombospondin-1 (TSP1) signaling axis in endothelial cells as critical for alveolar differentiation.[46] BMP4-Bmpr1a signaling caused calcineurin-NFATc1-dependent *Tsp1* expression in endothelial cells, leading to alveolus-specific differentiation of BASCs. In vivo, $Tsp^{-/-}$ mice had defects in alveolar repair following bleomycin injury.[46] Although studies with endothelial-specific knockout of *Tsp* are required, these data implicate endothelial-initiated signaling in epithelial cell differentiation of BASCs.

BMP4 was also recently implicated in reverse crosstalk. Deletion of matrix Gla protein (MGP), a BMP antagonist, resulted in vascular overgrowth and underdeveloped airways.[47] Ectopic differentiation of lung endothelium to a hepatic phenotype in $Mgp^{-/-}$ mice was inhibited by endothelial-specific knockout of the hepatic growth factor gene (*Hgf*).[48] Since BMP4 induces MGP, hence feedback inhibition of BMP4 activity, loss of MGP induces unchecked activation of BMP4. The resulting upregulation of endothelial HGF induces hepatic differentiation in $Mgp^{-/-}$ mice. These findings suggest that conditions that cause loss of MGP may increase activity of the BMP4-HGF pathway to cause dysfunctional differentiation of capillaries, as also of alveoli. However, the relevant capillary–alveolar crosstalk mechanisms require definition.

Conclusions

The study of cell–cell communication in the alveolar–capillary unit presents significant challenges, as cellular crosstalk is difficult to replicate in cell culture. In HPV and ALI models, significant progress has been made with the use of RFI. This work has implicated diffusible molecules, including ATP, arachidonate, and H_2O_2 in mediating forward and reverse signaling between alveoli and capillaries (Fig. 3). RFI studies suggest an absence of direct communication from capillaries and alveoli in mechanisms underlying ALI,[9] although immune cell recruitment initiated by endothelium appears essential for barrier loss. However, it remains unknown how systemic inducers of ALI, such as hemorrhagic shock or sepsis, initiate capillary–alveolar signaling to establish the known activation of alveolar macrophages.[49] The use of cell-specific knockouts has implicated capillary to alveolar signaling in lung development and regeneration. Further studies may reveal the mechanisms by which alveoli and capillaries carry out bidirectional crosstalk signaling to establish lung homeostasis and defense during development and pathogen challenge.

Conflict of interest

The author(s) declare that there is no conflict of interest.

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