Endothelial and epithelial signaling in the lung

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Chatterjee S, Baeter S, Bhattacharya J. Endothelial and epithelial signaling in the lung. Am J Physiol Lung Cell Mol Physiol 293: L517–L519, 2007. First published June 1, 2007; doi:10.1152/ajplung.00202.2007.—In recent years, pulmonary research has focused increasingly on the understanding of proinflammatory endothelial and epithelial signaling pathways underlying lung injury that is well known to be associated with high morbidity and mortality. Although traditionally considered separately, current research shows considerable convergence in epithelial and endothelial signaling mechanisms. This was well emphasized in the featured topic session held during the Experimental Biology 2007 Meeting in Washington, D.C., that addressed the complex interdependence between signaling pathways in the two cellular phenotypes. Several perspectives on endothelial and epithelial signaling, as well as their contributions to pulmonary inflammation and damage, as presented and discussed in the session are summarized here.

RBC INDUCE ENDOTHELIAL ROS IN HYPOXIA1

Although hypoxia causes lung inflammation that might lead to lung injury, underlying hypoxia-induced mechanisms are not known. The findings of this study indicated that reactive oxygen species (ROS) produced by red blood cells (RBC) might play a role in hypoxia-induced lung injury. Real-time fluorescence imaging of lung microvessels revealed that in the presence of RBC-containing vascular perfusion, hypoxia increased endothelial ROS and cytosolic Ca2+. These increases led to a proinflammatory response as evident in P-selectin-dependent leukocyte recruitment. However, in the presence of RBC-free perfusion, all hypoxia-induced responses were completely inhibited. Similar inhibitions were also evident when perfusion was carried out by RBC previously treated with inhibitors of hemoglobin (Hb) autoxidation, namely, carbon monoxide (CO) or nitrite, or together with the H2O2 inhibitor, catalase. By contrast, perfusion with RBC from BERK-trait mice, in which hypoxia augments RBC ROS production, enhanced the hypoxia-induced responses. The authors concluded that in hypoxia, increased Hb autoxidation augments ROS production in RBC. As a consequence, RBC release H2O2 that diffuses to the lung microvascular endothelium, thereby initiating Ca2+-dependent proinflammatory responses. In summary, RBC-derived ROS contributes to hypoxia-induced lung inflammation.

FLUORESCENCE IMAGING OF ENDOTHELIAL CONNEXIN43-MEDIATED PROINFLAMMATORY SIGNALING IN THE LUNG2

Spatially extensive inflammation is a major feature of acute lung injury. The extensive spread of inflammation that occurs across the lung’s surface rapidly increases inflammation throughout the lung and may thus be responsible for the mortality associated with acute respiratory distress syndrome. According to Parthasarathi et al. (12), intercellular communication has a role in the spread of this inflammation. Evidence presented in this paper showed that this communication is mediated by connexin43 gap junctions. Fluorescence imaging of intact lung microvessels was performed using the technique of photolytic uncaging of caged Ca2+ foci in alveolar capillaries. This increased endothelial Ca2+ in the target capillary as well as in adjacent venules. A peptide inhibitor of the intercellular gap junction protein, connexin43, completely blocked the communicated Ca2+ response. Moreover, Ca2+ communication was not evident in mice lacking endothelial connexin43. Connexin43-dependent Ca2+ communication increased production of ROS and expression of P-selectin in venules. Moreover, inhibition of connexin43 blocked thrombin-induced increases in lung microvascular permeability. The authors suggest that this novel role for connexin43-mediated gap junctions as channels or conduits for the spread of proinflammatory signals in the lung capillary bed can help in developing strategies to combat acute lung injury.

HIGH VASCULAR PRESSURE LEADS TO P450 EPOXYGENASE-DEPENDENT TRPV4 ACTIVATION AND ENDOTHELIAL INJURY IN MOUSE LUNG3

TRPV4, a member of the vanilloid subfamily of transient receptor potential channels, is a Ca2+-permeant channel regulated by shear stress, hypotonic cell swelling, 4α-phorbol-12,13-didecanoate (4αPDD), and epoxyeicosatrienoic acids (EETs) (7, 8, 14). In systemic endothelium, Ca2+ entry subsequent to shear stress requires EET-dependent gating of TRPV4 (7). This study considered whether TRPV4 was responsible for the Ca2+ entry-dependent permeability response in lung to another type of mechanical stress-radial stretch due

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to high vascular pressure (HiPv) (5). In lungs isolated from TRPV4+/− mice, the HiPv-induced permeability response was attenuated with low Ca2+ perfusate, pretreatment with the TRPV antagonist ruthenium red, and by pharmacological blockade of phospholipase A2 or P450 epoxidegenerases. In lungs from TRPV4−/− littermates, HiPv had little impact on permeability. This group has previously shown that in lung, pharmacological activation of TRPV4 with 4ePDD or EETs leads to disruption of alveolar septal endothelium and alveolar flooding (1). Together, the findings presented at the session in HiPv suggest that TRPV4 could play a clinically relevant role in acute lung injury scenarios that lead to dysfunction of the alveolar septal barrier.

DELETION OF CX40 COMBINED WITH CONDITIONAL DELETION OF ENDOTHELIAL CELL CX43 INDUCES LUNG EDEMA AND LUNG FIBROBLAST PROLIFERATION

This work tested the hypothesis that heterocellular communication via gap junctions between capillary endothelium, alveolar epithelium, and possibly fibroblasts may contribute to lung function. Isakson et al. (4) have demonstrated earlier that in mouse, the gap junction proteins connexin (Cx) 40 and Cx43 are found in alveolar type II (ATII) cells, and Cx43 is only found in capillary endothelium. For this reason, they created double-knockout mice formed from a global deletion of Cx40 and a conditional deletion of Cx43 from the vascular endothelial cells (VEC). These mice were found to have progressively hemorrhagic lungs that eventually led to a high number of animal deaths at ~35 wk. Upon examination of the terminal alveoli from C57, Cx40, VEC Cx43, and double-knockout mice at 8, 16, 24, and 32 wk of age, the double-knockout mice were found to have disorganized alveoli and disrupted septation. Using stains for elastin and collagen, evidence was provided of an increase in extracellular matrix deposition over the course of 32 wk in the double-knockout mice. The 32-wk double-knockout mice also had significantly higher cell numbers that correlated with an increase in staining for fibroblasts. This paper thus concluded that ATII cells, fibroblasts, and capillary endothelium may be linked via Cx40 and Cx43, and the loss of this connection may induce fibroblast proliferation and further extracellular matrix deposition. Together, this evidence implicates connexins as an important component to pathological lung fibrosis.

PARADOXICAL RESPONSE OF ENDOTHELIAL ROS PRODUCTION IN PEROXIREDOXIN 6 NULL MICE TO ISCHEMIA

The lung antioxidant enzyme peroxiredoxin 6 (Prdx6) has been shown to play a major role in antioxidant defense. This paper examined the effect of ischemia in Prdx6 null mice lungs using a model of normoxic lung ischemia, where ischemia implies stop of perfusate flow alone as lung ventilation during the ischemic period ensures normoxia. We have previously observed that in isolated perfused lungs, abrupt cessation of perfusate flow results in activation of endothelial cell NADPH oxidase, leading to the generation of ROS (9). In lung and heart injury models, Prdx6 null mice have hitherto been found to exhibit significantly greater oxidative damage compared with wild-type mice (10, 15). We thus hypothesized that in mice with deletion of Prdx6 by gene targeting, oxidative stress with ischemia would be significantly increased. Using amplex red for monitoring ROS generation in the lung endothelium by fluorescence microscopy, we observed that, contrary to expectation, ROS generation and oxidative damage with ischemia were significantly lower in Prdx6 null mice compared with wild-type mice. Quantification of the images showed a 100% increase of endothelial ROS after 1 min of ischemia in wild-type lungs, whereas in Prdx6−/− lungs, the increase was a modest 15%. Stimulated alveolar macrophages from wild-type mice showed greater ROS generation compared with Prdx null mice. As the lung ischemia model of injury involves ROS generation through NADPH oxidase activation, the abrogation of ROS with ischemia in Prdx6−/− is compatible with the involvement of Prdx6 in NADPH oxidase activation.

ACTIVATION OF TOLL-LIKE RECEPTOR 4 IS A CRITICAL DETERMINANT OF LUNG NEUTROPHIL SEQUESTRATION AND INJURY INDUCED BY HIGH TIDAL VOLUME MECHANICAL VENTILATION

The infiltration of polymorphonuclear neutrophils (PMNs) into lungs is an important feature of ventilator-induced lung injury associated with pneumonia, but the mechanisms that recruit PMNs are poorly understood. Using Toll-like receptor 4 knockout mice (TLR4−/−), this presentation addressed the possible role of TLR4 signaling in PMN recruitment. Wild-type or TLR4−/− mice were challenged by intratracheal instillation of LPS (3.0 mg/kg) for 2 h and then subjected to high tidal volume mechanical ventilation (28 ml/kg) for 2 h. Ventilated wild-type mice exhibited significant increases in PMN sequestration, transvascular protein permeability, and edema formation. Quantitative lung histopathology demonstrated PMN infiltration, alveolar hemorrhage, edema, and alveolar wall thickening in lungs of ventilated/LPS-challenged wild-type mice. However, TLR4−/− mice showed negligible PMN sequestration, microvascular barrier breakdown, and edema formation. Thus high tidal volume ventilation during pneumonia induces PMN sequestration and lung injury via TLR4-dependent signaling pathways. These results suggest the important role of lung innate immunity mechanisms as regulated by TLRs in mediating PMN sequestration and injury resulting from mechanical ventilation at high tidal volumes.

DYSFUNCTION OF CFTR IN ALVEOLAR MACROPHAGES AND NEUTROPHILS PROPAGATES PRODUCTION OF PROINFLAMMATORY CYTOKINES AND EXACERBATES ACUTE LUNG INJURY IN MICE

CFTR plays a major role in alveolar fluid clearance in a mouse model of acute volume-overload pulmonary edema by a cAMP-dependent mechanism (3). There is new evidence that

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both alveolar type I and II cells express CFTR (6). In airway epithelial cells, inhibition of CFTR can worsen the proinflammatory profile by increasing IL-8 production and promoting NF-κB p65 translocation (13). Recent studies have shown that human and murine alveolar macrophages express CFTR, which contributes to phagolysosomal acidification (2). Human neutrophils express CFTR, which promotes chlorination (11). Dysfunction of CFTR in the phagolysosome leads to reduction of acidification capacity and bacterial killing ability. Dr. Su and colleagues found that ΔF508 mice have an exaggerated inflammatory response to LPS in the lung. The CFTR inhibitor MaH-2 worsened pulmonary edema and increased macrophage inflammatory protein-2 (MIP-2) levels in the lung homogenate in LPS-induced acute lung injury. Therefore, they hypothesized that inhibition and mutation of CFTR in the alveolar macrophages and neutrophils would contribute to exaggerated lung inflammation and worsen acute lung injury. Dr. Su and colleagues further discovered that alveolar macrophages and neutrophils expressed CFTR, demonstrated by immunofluorescence and Western blot. Under LPS simulation, CFTR expression in alveolar macrophages and neutrophils was increased. Inhibition and mutation of CFTR in the LPS-simulated alveolar macrophages and neutrophils increased MIP-2 production by an NF-κB-dependent mechanism. In the LPS-induced acute lung injury mouse model, inhibition and mutation of CFTR increased lung endothelial and epithelial permeability furthered by an increase of protein concentration in the bronchoalveolar lavage. These results suggest that impaired CFTR function increases pulmonary edema by two mechanisms: lack of functional CFTR impairs cAMP-dependent alveolar fluid clearance, and dysfunction of CFTR increases the proinflammatory responses and probably worsens pulmonary edema.

NO-REGULATED NEGATIVE FEEDBACK LOOP PROTECTS LUNG ENDOTHELIAL BARRIER FUNCTION IN HYDROSTATIC STRESS

Although the formation of hydrostatic lung edema as a result of increased lung microvascular pressure has previously been attributed almost exclusively to an imbalance of Starling forces, active endothelial responses to mechanical stress may contribute importantly to this scenario. With the use of intravital fluorescence imaging and microgravimetric techniques, lung capillary endothelial cells were found to respond to increased hydrostatic stress with an increase in cytosolic Ca2+ concentration, which increased endothelial permeability (K̇i) via activation of myosin light chain kinase (MLCK). Simultaneously, hydrostatic pressure activates endothelial NO synthase via both Ca2+ and PI3K-dependent mechanisms. The resulting NO formation diminishes the increase in K̇i by attenuating the pressure-induced Ca2+ influx in a cGMP-dependent manner. Pharmacological inhibition by both Gd3+ and ruthenium red suggests the mechanosensitive Ca2+ channel in lung endothelial cells to be TRPV4, and cGMP-dependent regulation of TRPV4 was confirmed by both intravital imaging and patch-clamp experiments. These data provide the first evidence for the regulation of TRPV4 by cyclic nucleotides. The present findings identify a complex scenario of endothelial responses to hydrostatic stress in which the deterioration of the lung microvascular barrier by a Ca2+-dependent activation of MLCK is effectively counteracted via an intrinsic NO/cGMP-regulated negative feedback loop.

In summary, the session added to our understanding of the multiple signaling molecules and pathways that can lead to lung injury. The session highlighted the understanding that numerous factors such as ROS/NO, connexins, TRPV4, TLR4, and CFTR variously regulate lung inflammatory and injury processes. Further studies are required to elucidate the interactions among these factors during lung injury and repair.

REFERENCES


* Presented by J. Yin (J. Yin, H. Kuppe, and W. M. Kuebler, Charité Medical University and German Heart Institute, Berlin, Germany).