Loss of endothelial CFTR drives barrier failure and edema formation in lung infection and can be targeted by CFTR potentiation

Lasti Erfinanda[1], Lin Zou[1,2,3], Birgitt Gutbier[4], Laura Kneller[4], Sarah Weidenfeld[1], Laura Michalick[1], Disi Lei[1,2], Katrin Reppe[4], Luiz Gustavo Teixeira Alves[4], Bill Schneider[4], Qi Zhang[1], Caihong Li[1], Diana Fatykhova[4], Paul Schneider[5], Wolfgang Liedtke[6], Eisei Sohara[7], Timothy J. Mitchell[8], Achim D Gruber[9], Andreas Hocke[4,10,11], Stefan Hippenstiel[4,10,11], Norbert Suttorp[4,10,11], Andrea Olschewski[12], Marcus A. Mall[11,13], Martin Witzenrath[4,10,11], Wolfgang M. Kuebler[1,11*]

[1]Inst. Physiology., Charité Berlin, Germany, [2]German Heart Center, Berlin, Germany, [3]Dept. of Endocrinology, Shanghai Pudong New Area Gongli Hospital, Shanghai, China, [4]Div. of Pulmonary Inflammation, Charité Berlin, Berlin, Germany, [5]Dept. for General and Thoracic Surgery, DRK Clinics, Berlin, Germany, [6]Dept. of Neurology, Duke University Medical Center, Durham, USA, [7]Dept. of Nephrology, Graduate School of Medical and Dental Sciences, Tokyo Medical and Dental University, Japan, [8]Inst. of Microbiology and Infection, University of Birmingham, Birmingham, B15-2TT, UK, [9]Inst. of Veterinary Pathology, Freie Universität Berlin, Berlin, Germany, [10]Dept. of Inf. Diseases and Pulmonary Medicine, Charité Berlin, Berlin, Germany, [11]German Center for Lung Research (DZL), associated partner site, Berlin, Germany, [12]Experimental Anaesthesiology, Dept. of Anaesthesiology and Intensive Care Medicine, Medical University of Graz, Graz, Austria, [13]Dept. of Pediatric Pulmonology, Immunology and Intensive Care Medicine, Charité Berlin, Berlin, Germany.

Pneumonia is the most common cause of the acute respiratory distress syndrome (ARDS), a potentially fatal lung disease characterized by hyperinflammation and endothelial barrier failure. Infectious and inflammatory stimuli can cause rapid downregulation of cystic fibrosis transmembrane conductance regulator (CFTR), and inhibition of CFTR was found to increase lung microvascular endothelial permeability *in vitro*. Here, we identify loss of lung microvascular endothelial CFTR as important pathomechanism in lung endothelial barrier failure in pneumonia-induced ARDS, delineate the molecular signaling pathway underlying this effect and identify CFTR potentiation as novel therapeutic strategy in ARDS.

CFTR was downregulated following *Streptococcus pneumoniae* (*S.pn.*) infection in human and murine lung tissue. CFTR downregulation was also observed in human pulmonary microvascular endothelial cells (HPMECs) following infection with *Pseudomonas aeruginosa* or stimulation with pneumolysin (PLY), a virulence factor of *S.pn.*, or by plasma from COVID-19 patients. Isolated perfused lungs revealed that CFTR inhibition increased endothelial permeability in parallel with intracellular Cl^- and Ca^{2+} concentrations ($[Cl^-]_i$, $[Ca^{2+}]_i$). Inhibition of the Cl^- sensitive with-no-lysine kinase 1 (WNK1) replicated the effect of CFTR inhibition on endothelial permeability and endothelial $[Ca^{2+}]_i$ while WNK1 activation attenuated it. Lungs of heterozygous Wnk1-deficient mice (*Wnk1*$^{+/-}$) showed spontaneous leak. Endothelial $[Ca^{2+}]_i$ transients and permeability in response to inhibition of either CFTR or WNK1 were prevented by inhibition of the cation channel transient receptor potential vanilloid 4 (TRPV4). Mice deficient in *Trpv4* (*Trpv4*$^{-/-}$) developed less lung edema and protein leak than their wild-type littermates following infection with *S. pn.* The CFTR potentiator ivacaftor prevented CFTR loss and reduced endothelial leak in response to PLY or plasma from COVID-19 patients *in vitro*, and prevented lung edema and protein leak after *S. pn.* infection *in vivo*.

Lung infection causes rapid loss of CFTR that promotes lung edema formation through intracellular Cl^- accumulation, inhibition of WNK1 and subsequent disinhibition of TRPV4, resulting in endothelial Ca^{2+} influx and vascular barrier failure. Ivacaftor prevents CFTR loss and may thus present a promising therapeutic strategy in ARDS due to severe pneumonia including COVID-19.

Supported by the German Research Foundation (CRC-TR84, C09).