Int J Clin Exp Pathol 2018;11(6):2959-2967
www.ijcep.com /ISSN:1936-2625/IJCEP0074619

Original Article

Lipopolysaccharides increase Kir2.1 expression in lung endothelial cells

Wei Fang[1*], Lin Xia[2*], Xiaoxia Sun[1], Shixia Cai[1], Suwen Bai[2], Yunbo Sun[1], Peihong Zhou[1], Xin Liu[1], Ren Zhao[3], Bing Shen[2]

[1]Department of ICU, The Affiliated Hospital of Qingdao University, Qingdao, Shandong, China; [2]School of Basic Medical Sciences, Anhui Medical University, Hefei, Anhui, China; [3]Department of Cardiology, The First Affiliated Hospital of Anhui Medical University, Hefei, Anhui, China. [*]Equal contributors.

Received February 16, 2018; Accepted March 24, 2018; Epub June 1, 2018; Published June 15, 2018

Abstract: Kir2.1 is an inwardly rectifying K^+ channel that modulates membrane potential. It is expressed widely in smooth muscle, neurons, and endothelial cells. Acute lung injury (ALI) and acute respiratory distress syndrome (ARDS) are severe clinical syndromes, often causing the damage of epithelial and endothelial cells. Lipopolysaccharides (LPS) usually cause ALI/ARDS, directly or indirectly, and are used to reproduce the model *in vivo*. Here, we used differentially expressed gene analysis to find increasing Kir2.1 channel expression in human pulmonary microvascular endothelial cells cultured with LPS. Primary cultured mice pulmonary microvascular endothelial cells were verified by immunofluorescence. LPS incubation increased Kir2.1 channel expression in cultured mice pulmonary microvascular endothelial cells. A whole-cell voltage clamp was used to record the K^+ current in cultured endothelial cells, showing increased whole-cell current in LPS treatment compared with controls. Additionally, the application of Ba^{2+}, as an inhibitor of Kir2.1 channel, inhibited K^+ current in both groups. We demonstrated that LPS may increase Kir2.1 channel expression in mice pulmonary microvascular endothelial cells to increase K^+ flux, maintain hyperpolarization, and cause vasodilation, which may increase blood flow in pulmonary vessel bed, leading to pulmonary congestion contributing pneumonemia and ALI/ARDS.

Keywords: Lipopolysaccharides, Kir2.1, acute lung injury, acute respiratory distress syndrome, endothelial cells

Introduction

Acute lung injury (ALI) and acute respiratory distress syndrome (ARDS) are severe clinical syndromes characterized by damage to epithelial and endothelial cells, furthering pulmonary edema in alveoli and excessive pulmonary inflammation, finally resulting in the disruption of pulmonary gas exchange [1]. ARDS often causes acute hypoxemic respiratory failure and bilateral pulmonary infiltrates, leading to interstitial and alveolar pulmonary edema which is not attributed to left atrial hypertension [2]. ALI/ARDS contributes to high mortality and specific therapies need to be uncovered [1].

Lipopolysaccharides (LPS), important constituents of gram-negative bacterial cell walls, can injury pulmonary microvascular walls and accumulate leukocytes to damage lung epithelial cells [3]. Impaired epithelial cells lose the ability to clear alveolar fluid and produce pulmonary edema. LPS usually causes ALI/ARDS, directly or indirectly, leading to pneumonia, sepsis, and even chronic disorders [4]. In previous studies of ALI/ARDS, LPS was often used to reproduce the ALI/ARDS model *in vivo* [4]. Pulmonary edema and alveolar flooding have been chosen as hallmarks of ALI/ARDS in animal models [5]. Hence, pulmonary endothelium is of great importance in LPS-induced ALI/ARDS because it determines permeability of the alveolar-capillary barrier [6]. Impaired endothelium can lead to over-exudation of protein-rich fluids in alveolar space and further pulmonary edema. Other kinds of cells also participate in ALI/ARDS, including epithelial cells and leukocytes. Epithelial cells usually exert a protective function resistant to environmental hazards and induce over inflammatory responses via NF-κB and MAPPK pathways [7, 8]. Inflammation caused by neutrophils is characteristic in human ALI.

Inward rectifier K^+ channels (Kir), a kind of ion channel family, are transcribed from *KCNJ* genes family and are expressed widely on endothelial cell membranes, including Kir2.1 to Kir2.4 [9]. These K^+ channels consist of two membrane spanning domains to form a tetramer with intracellular carboxyl and amino terminals [10]. Previous studies have reported that Kir2.1 interacts with postsynaptic density proteins (PSD) via PDZ domain recognition sequence locating at the carboxyl terminal of Kir2.1 [11]. Most K^+ channels allow an inward K^+ current when the membrane potential is negative to K^+ equilibrium potential and allow an outward K^+ current for a membrane potential positive to its equilibrium potential. However, it is different that inward rectifier K^+ channels allow a normal inward K^+ current but a restrictive and small outward K^+ current when membrane potential is between K^+ equilibrium potential and resting membrane potential. This small outward K^+ current reinforces hyperpolarization of cell membranes, the most important function of Kir channels [12, 13].

Meanwhile, Kir2.1 channels are found to be present in macro-vascular endothelial cells and are activated by fluid shear stress for vasodilation through modulation of membrane potential and ions exchange [14, 15]. This mechanism, however, has not been proven among microvascular endothelial cells, although some previous studies have found that Kir2.1 is an essential subunit of native microvascular endothelial cells from rats and mice. One study elucidated that Kir2.1 channels transduce a small extracellular K^+ increasing into a vasodilation signaling cerebral capillaries [15]. Another study showed that brain capillary endothelial cells, under hypoxic conditions, expressed more Kir2.1 channels accompanying with hyperpolarization and enhanced store-operated Ca^{2+} entry [16]. Additionally, this Kir2.1 related-hyperpolarization has been proven in renal vascular endothelial cells [17].

Based on previous studies and our data from differentially expressed gene (DEGs) analysis, we hypothesized that LPS may induce increasing Kir2.1 channels in pulmonary endothelial cells and hyperpolarization of endothelial cells in pulmonary micro vessels and contribute to ALI/ARDS. In the present study, we performed Western blot, immunofluorescence, and patch-clamp techniques to elucidate whether LPS increases Kir2.1 channels and further induces hyperpolarization in pulmonary endothelial cells.

Methods and materials

Microarray data

The transcription profile of GSE5883 was downloaded from NCBI GEO database (Gene Expression Omnibus, http://www.ncbi.nlm.nih.gov/geo/). Data included 4 samples from human pulmonary microvascular endothelial cells cultured with 10 ng LPS for 4 h (GSM114552, GSM114553, GSM114554, GSM114555), 4 samples for 8 h (GSM114556, GSM114557, GSM114558, GSM114559), and 4 samples for 24 h (GSM114560, GSM114561, GSM114562, GSM114563).

Cell isolation and culture

All animal experiments were performed according to NIH publication no. 8523 and were approved by the Animal Experimentation Ethics Committee of Anhui Medical University. Pulmonary microvascular endothelial cells were isolated from C57 mouse lung tissues and maintained in Dulbecco's modified Eagle's medium (DMEM) at 37°C in an incubator with 5% CO_2. To verify isolated cells, we performed immunofluorescence assay to detect whether VE-Cadherin, vascular endothelial growth factor (VEGF), and FLK-1 proteins were expressed in isolated cells. The culture medium for LPS group contained 10 ng/mL LPS. The following studies were performed on cells treated with LPS for 4 hours, 8 hours, or 24 hours. Phosphate-buffered solution (PBS) was used as a solvent control.

Western blotting

Western blotting was performed, as previously described [18]. Cultured pulmonary microvascular endothelial cells were trypsinized and followed by 3 washings with ice-cold PBS. Cells were then incubated with 500 μL ice-cold freshly prepared lysis buffer (20 mM Tris-HCl, 150 mM NaCl, 1 mM EGTA, 0.5% Triton X-100, pH 7.3). Following centrifugation at 12,000 rpm for 30 minutes at 4°C, supernatant was collected and protein concentration was determined by Bradford assay. After 30 μg proteins were loaded in each well of a 10% SDS-PAGE

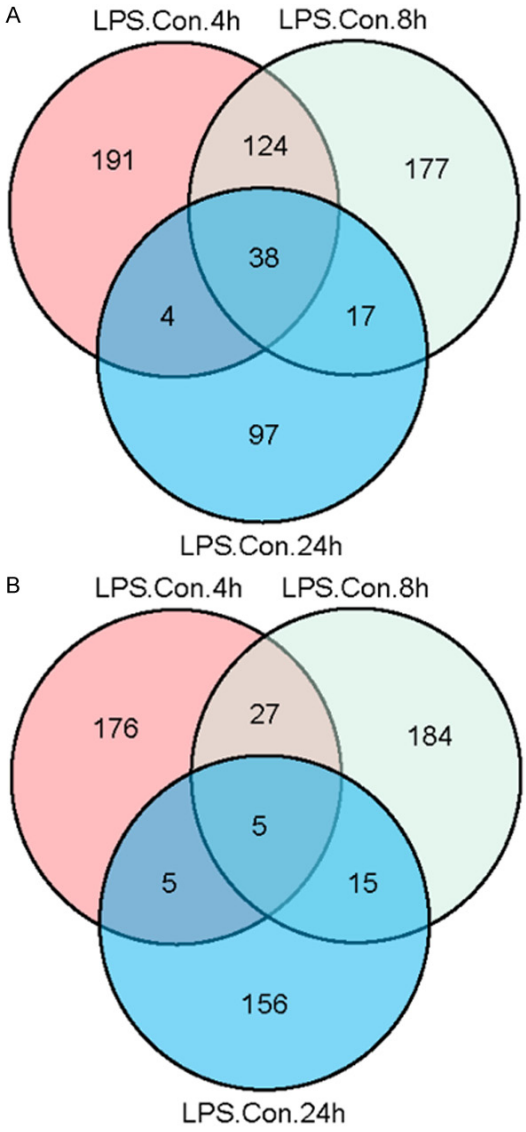

Figure 1. Venn diagram illustration of gene expression similarity. A. Upregulated genes in human pulmonary microvascular endothelial cells cultured with lipopolysaccharide (LPS, 10 ng/mL) for 4 h, 8 h, or 24 h. 38 genes were upregulated in all time groups. B. Downregulated genes in human pulmonary microvascular endothelial cells cultured with LPS for 4 h, 8 h, or 24 h. 5 genes were downregulated in all three times groups.

gel, the proteins were transferred to a polyvinylidene difluoride membrane. Membranes carrying proteins were first incubated with TBST containing 10% non-fat dried milk for 1 hour at room temperature, to block non-specific antigens. For immunoblots, the membrane containing transferred proteins was incubated with anti-Kir2.1 antibody (1:200, rabbit polyclonal, Bioss company) overnight at 4°C. Then, the membrane was incubated with horseradish peroxidase-conjugated secondary antibody for 2 hours at room temperature. Immunosignal resulting was detected using an ECL detection system. Optical intensity of the protein bands was normalized to GAPDH on the same lane and presented as relative optical density.

Immunofluorescence

Pulmonary microvascular endothelial cells were grown on poly-L-lysine-coated coverslips in serum-free DMEM, for 24 hours at 37°C, in an incubator with 5% CO_2. Then, pulmonary microvascular endothelial cells were fixed in a 4% paraformaldehyde solution for 25 minutes at 25°C. For antigen retrieval, coverslips were heated with citrate buffer at 95°C for 10 minutes in a microwave oven. After 3 washings, the pulmonary endothelial cells were permeabilized by a solution of 0.1% Triton X-100 and 0.1% sodium citrate for 5 minutes at room temperature. After blocking the unspecific antigen, pulmonary endothelial cells in the slice were treated with diluted anti-VE-Cadherin, anti-VEGF, anti-FLK-1, or anti-Kir2.1 antibody (1:200, rabbit polyclonal, Bioss company) in 1% bovine serum albumin (BSA) and PBST in a humidified chamber overnight at 4°C. Pulmonary microvascular endothelial cells were then incubated with the secondary antibody in 1% BSA for 1 hour at room temperature, in the dark, and washed 3 times with PBS for 5 minutes each in the dark. Finally, pulmonary microvascular endothelial cell nuclei were stained with 4',6-diamidino-2-phenylindole (DAPI). All images were acquired using Leica TCS SP5 confocal microscope system. The total pulmonary microvascular endothelial cell nuclei were recorded as blue dots at a wavelength of 460 nm and various proteins were recorded as green dots at a wavelength of 520 nm.

Electrophysiological recording

A whole-cell voltage clamp was performed using an EPC-9 amplifier (HEKA, Germany) at room temperature (22-24°C), as previously described [19]. The holding potential was at -60 mV and voltage ramp was from -100 mV to +100 mV over a duration of 500 ms. Patch pipette had a resistance of 2-5 MΩ. The standard bath solution consisted of (in mM): 140 NaCl, 5 KCl, 1 $CaCl_2$, 1 $MgCl_2$, 10 glucose, 5 HEPES, and pH 7.3 adjusted with NaOH. The

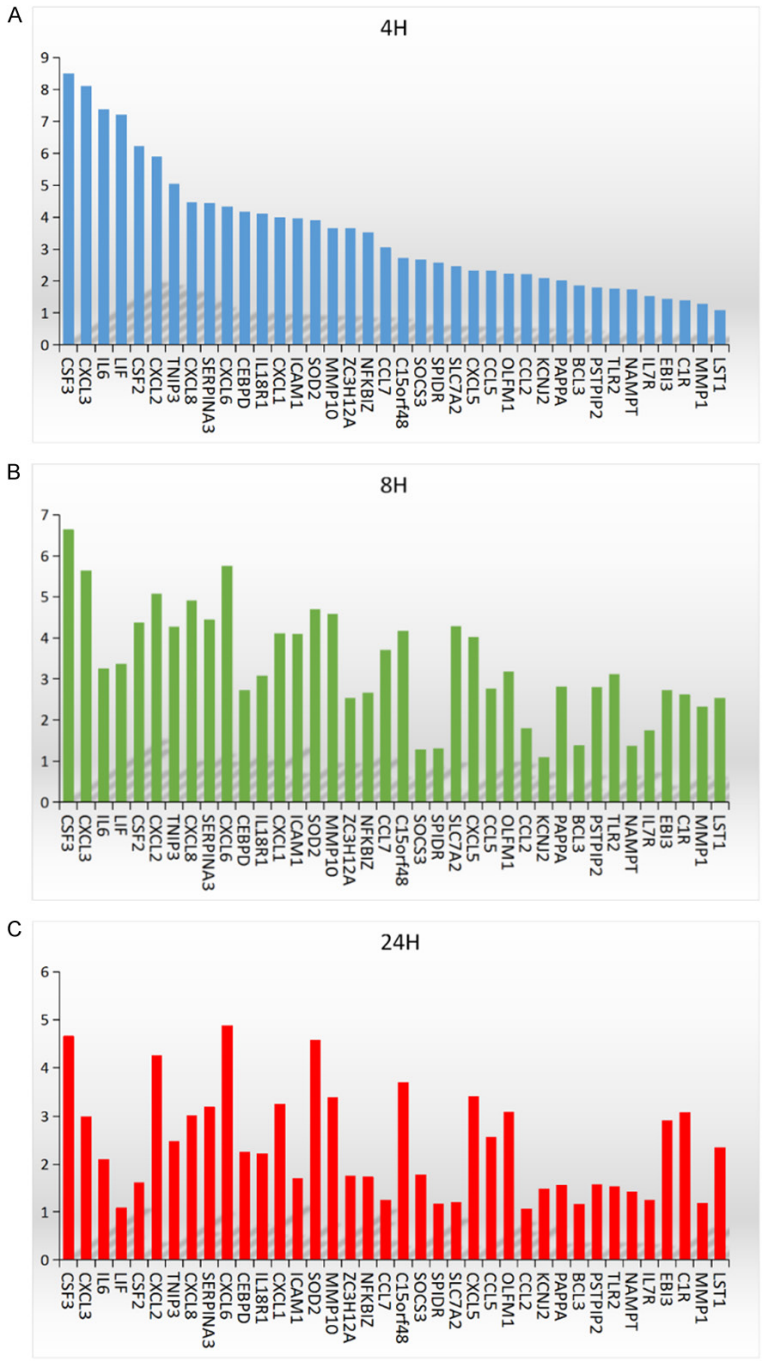

Figure 2. Fold changes (expressed as |log (fold change)|) of significant ($P < 0.05$) upregulated 38 genes in all three time (4 h, 8 h, and 24 h) groups in human pulmonary microvascular endothelial cells cultured with lipopolysaccharides (LPS, 10 ng/mL) for 4 h, 8 h, or 24 h.

pipette solution contained (in mM): 110 K-Glutamine, 35 KCl, 2 $MgCl_2$, 1 $CaCl_2$, 10 EGTA, 10 HEPES, and pH 7.3 adjusted with KOH. Data analysis was performed using Patchmaster software.

Statistical data analysis

SigmaPlot software was used to conduct unpaired Student's *t*-tests. All results are expressed as mean ± SEM. Current-voltage curves in electrophysiology were representative of recordings from multiple experiments. Student's *t*-test was used for statistical comparison and $P < 0.05$ was considered a statistically significant difference.

Results

Identification of DEGs

Microarray expression profiling from GEO database was used to identify significant DEGs in human pulmonary microvascular endothelial cells cultured with LPS for 4 hours, 8 hours, or 24 hours. There were 357 upregulated and 213 downregulated DEGs in 4 hour group, 356 upregulated and 231 downregulated DEGs in 8 hour group, and 156 upregulated and 181 downregulated DEGs in 24 hour group. However, we found that 38 upregulated and 5 downregulated DEGs from 4 hour group also existed in 8 hour and 24 hour groups (**Figure 1**). The top 10 upregulated in the 4 hour, 8 hour, and 24 hour groups DEGs are shown in **Figure 2**.

From the 38 upregulated DEGs in all 3 groups, we identified that *KCNJ2* gene was a key element. Its expression was increased 4.2, 2.1, and 2.8 fold at 4 hours, 8 hours, and 24 hours. *KCNJ2* transcribes and translates to Kir2.1 proteins and has been known as an inward rectifier K^+ channel related to membrane potential [9]. As described previously, Kir2.1 has been found to function in the vasodi-

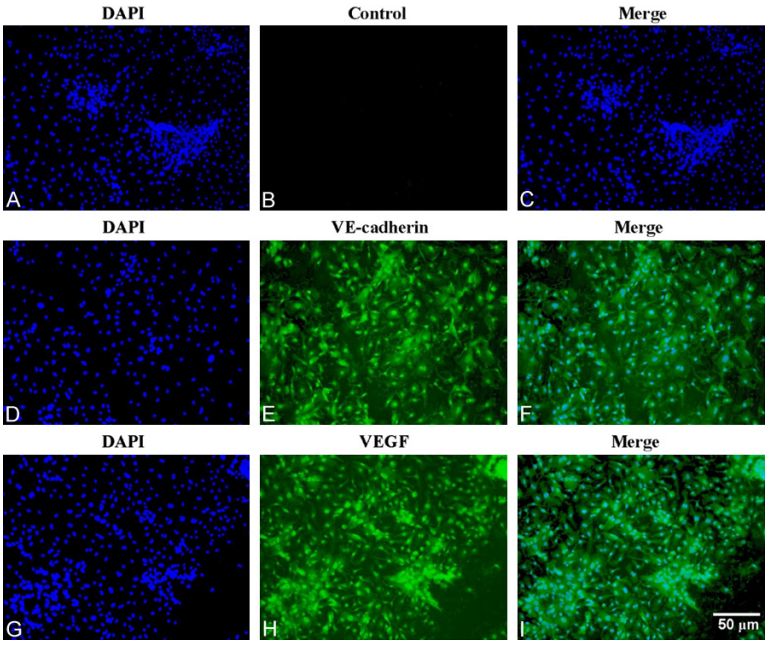

Figure 3. Identification of primary-cultured mice pulmonary microvascular endothelial cells. Representative immunofluorescence images show no primary antibody control (A-C), expression of VE-Cadherin (D-F, green), and microvascular endothelial growth factor (VEGF) (G-I, green). Nuclei (blue) were labeled by DAPI staining. Scale bar represents 50 μm.

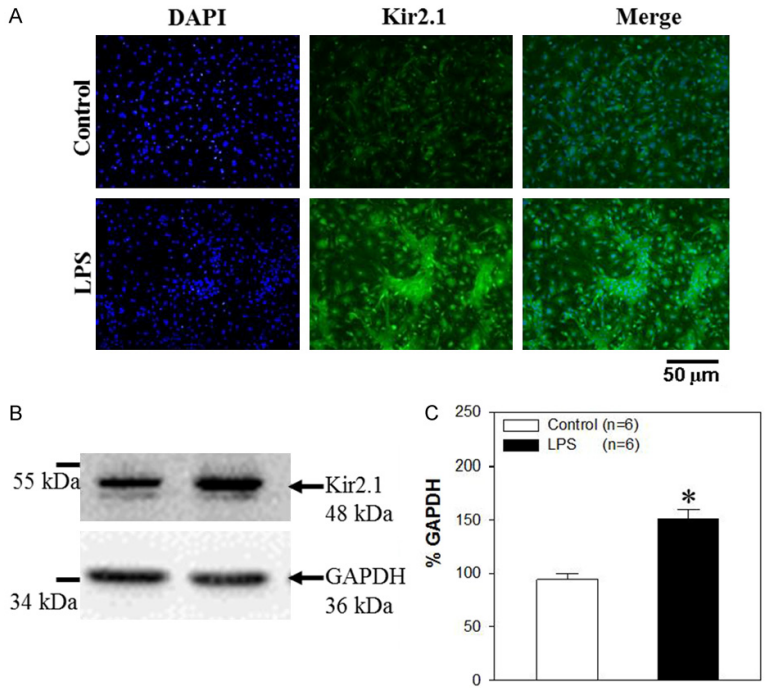

Figure 4. Llipopolysaccharides (LPS) effect on expression of Kir2.1. (A) Representative immunofluorescence images showing Kir2.1 (green) expression levels of primary-cultured mice pulmonary microvascular endothelial cells with saline control or LPS (10 ng/mL) treatment. Nuclei (blue) were labeled by DAPI staining. Scale bar represents 50 μm. (B, C) Representative immunoblot images (B) and summarized data (C) showing Kir2.1 expression levels of primary-cultured mice pulmonary microvascular endothelial cells with saline control or LPS (10 ng/mL) treatment. Protein expression was normalized with GAPDH. Protein levels are expressed as the relative optical density. Values are shown as mean ± SEM (n = 6 each group). $*P < 0.05$, control vs. LPS.

lation of cerebral capillaries and renal vascular endothelial cells [17, 20]. Hence, Kir2.1 is likely able to cause pulmonary vasodilation and contribute to pulmonary edema.

LPS increased Kir2.1 expression in pulmonary microvascular endothelial cells

To prove the function of Kir2.1 in LPS-induced pulmonary microvascular endothelial cells, we isolated and cultured mouse pulmonary microvascular endothelial cells *in vitro*. After isolation, we first performed immunofluorescence assay to verify the isolated cells. Cell nuclei were stained as blue fluorescence via DAPI assay. VE-Cadherin and VEGF were stained as green fluorescence via respective antibodies and expressed in isolated cells (**Figure 3**). This result showed that the isolated cells were pulmonary microvascular endothelial cells.

We then detected Kir2.1 proteins in pulmonary microvascular endothelial cells by immunofluorescence and Western blotting assay. Cell nuclei were stained as blue fluorescence via DAPI assay. Pulmonary microvascular endothelial cells were found to express Kir2.1 as green fluorescence and LPS obviously enhanced Kir2.1 expression (**Figure 4A**). To further identify Kir2.1 expression change, we used

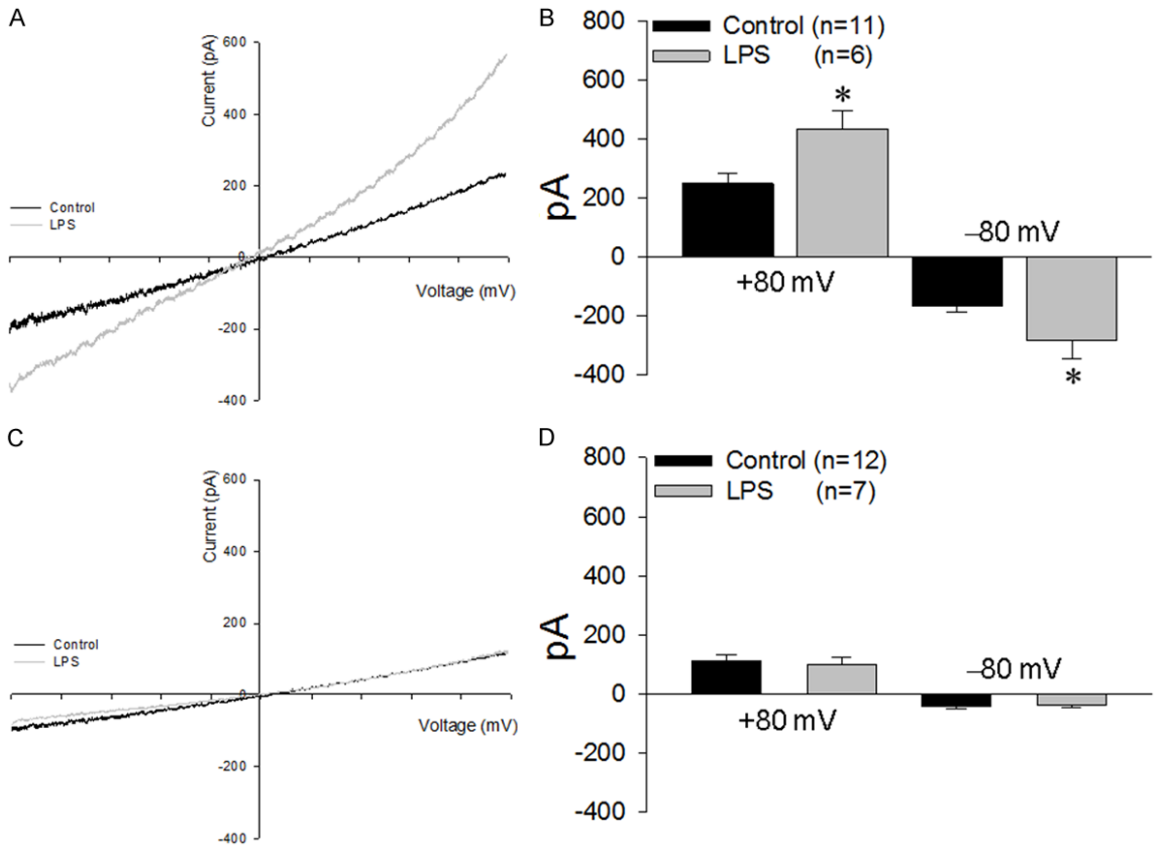

Figure 5. Llipopolysaccharides (LPS) effect on Kir2.1 whole-cell current. A, B. Representative *I-V* ramp traces and summarized data showing Kir2.1 whole-cell current in primary-cultured mice pulmonary microvascular endothelial cells with saline control or LPS (10 ng/mL) treatment. C, D. Representative *I-V* ramp traces and summarized data showing $BaCl_2$ (10 mM) blocking effect on Kir2.1 whole-cell current in primary-cultured mice pulmonary microvascular endothelial cells with saline control or LPS (10 ng/mL) treatment. Values are shown as mean ± SEM (n = 6-12 each group). $*P < 0.05$, control vs. LPS.

Western blotting to demonstrate that more Kir2.1 was expressed in pulmonary microvascular endothelial cells after incubation with LPS (**Figure 4B** and **4C**).

LPS reinforced Kir2.1 current in pulmonary microvascular endothelial cells

Because only Kir2.1 expressed on cell membranes is able to influence membrane potential, to test whether there were increasing Kir2.1 on cell membranes to mediate K^+ flux, we performed whole-cell voltage clamp to measure K^+ current in pulmonary microvascular endothelial cells. Compared with PBS control, LPS incubation significantly increased cell K^+ current with membrane voltage from -100 mV to +100 mV and -80 mV and +80 mV (**Figure 5A** and **5B**). This result indicates that LPS increases Kir2.1 current in pulmonary microvascular endothelial cells. To further verify Kir2.1 current, $BaCl_2$ (10 mM) was applied to block ion channels of Kir2.1 [16]. Our data shows that $BaCl_2$ dramatically inhibited this K^+ current in PBS or LPS treatment groups (**Figure 5C** and **5D**).

Discussion

Kir2.1, a member of KIR channel family, is an inwardly rectifying K^+ channel that modulates membrane potential. It is expressed widely in cardiac myocytes, neurons, and endothelial cells [21-23]. In this study, we proposed that increasing Kir2.1 is involved in LPS-induced ALI/ARDS. The evidence we found is as follows: (1) DEG analysis indicated LPS incubation for 4 hours, 8 hours, and 24 hours increased Kir2.1 expression in human microvascular pulmonary endothelial cells; (2) Mouse pulmonary micro-

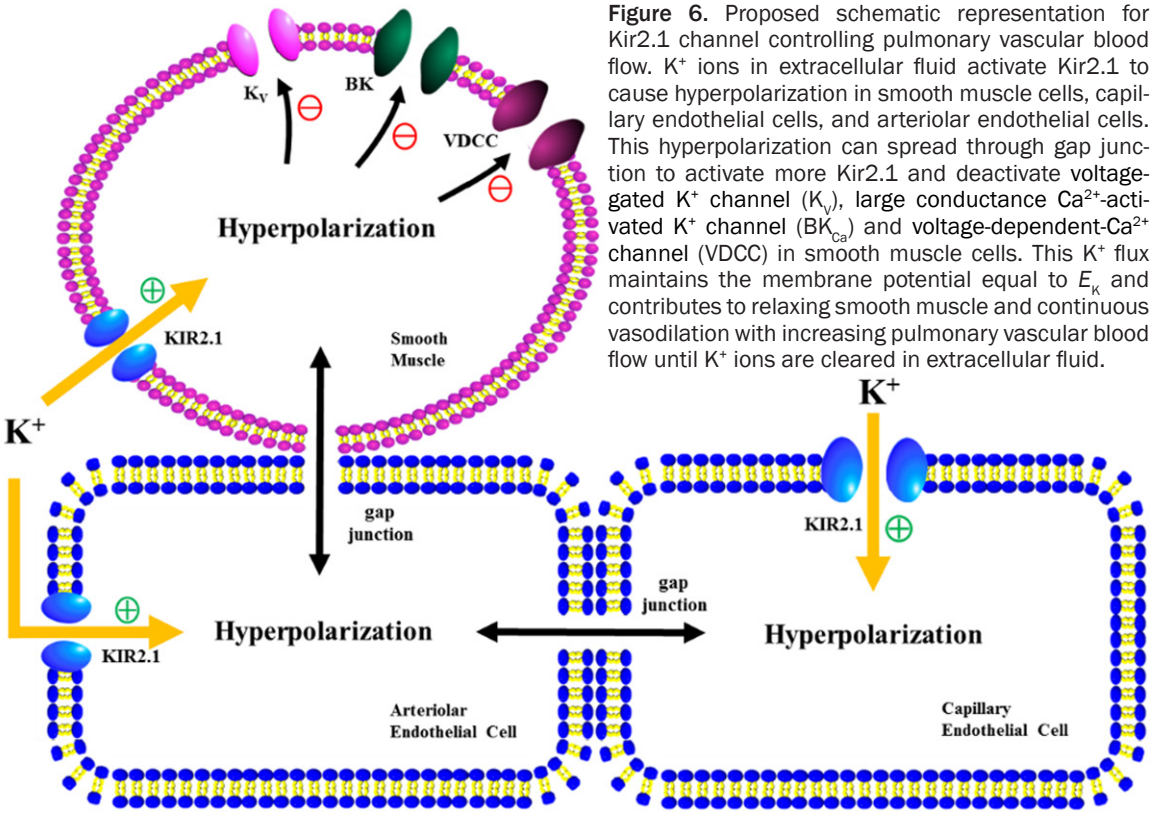

Figure 6. Proposed schematic representation for Kir2.1 channel controlling pulmonary vascular blood flow. K^+ ions in extracellular fluid activate Kir2.1 to cause hyperpolarization in smooth muscle cells, capillary endothelial cells, and arteriolar endothelial cells. This hyperpolarization can spread through gap junction to activate more Kir2.1 and deactivate voltage-gated K^+ channel (K_V), large conductance Ca^{2+}-activated K^+ channel (BK_{Ca}) and voltage-dependent-Ca^{2+} channel (VDCC) in smooth muscle cells. This K^+ flux maintains the membrane potential equal to E_K and contributes to relaxing smooth muscle and continuous vasodilation with increasing pulmonary vascular blood flow until K^+ ions are cleared in extracellular fluid.

vascular endothelial cells were isolated and verified via immunofluorescence; (3) Immunofluorescence and Western blotting assays showed increasing Kir2.1 expression in pulmonary microvascular endothelial cells after LPS treatment; (4) Increasing Kir2.1 K^+ current was demonstrated in pulmonary microvascular endothelial cells treated with LPS, which was blocked by Ba^{2+}.

In most cells, K^+ channels are responsible for modulation of membrane potential, ion exchange, and cell volume [24]. It has been proven that Kir2.1 functions to maintain cell resting membrane potential close to K^+ equilibrium potential [9]. Hence, the amount of Kir2.1 expressed in cells influences cell membrane potential. Activation of Kir2.1 channels can induce a hyperpolarization on either excitable or unexcitable cells. For excitable cells, it can cause a long-lasting potential plateau in cardiac myocytes, as well as a relaxation and further vasodilation in vascular smooth muscle cell [9]. For unexcitable cells, this membrane hyperpolarization becomes an electrical signaling transmitted to adjacent cells via electrical gap junctions, such as endothelial cell-smooth muscle (**Figure 6**) [20, 25]. Then, the adjacent smooth muscle cells become hyperpolarized and relaxed.

Previous studies have demonstrated that Kir2.1 is expressed in both vascular endothelial cells and smooth muscle cells [26, 27]. Kir channels have been considered as the most prominent channels in vascular endothelial cells. They can be blocked by Ba^{2+}, inhibiting Ca^{2+} influx, and further vasodilation [26, 28-30]. Meanwhile, Kir2.1 channels also cause hyperpolarization and relaxation in vascular smooth muscle cells and are blocked by Ba^{2+} [31]. In the present study, we analyzed NCBI GEO data and found that LPS treatment enhanced Kir2.1 expression in pulmonary microvascular endothelial cells at different time points. Moreover, LPS treatment induced increasing Kir2.1 current which can be blocked by Ba^{2+} in pulmonary microvascular endothelial cells. Based on previous knowledge, we used an endothelial cell-smooth muscle cell coupling model (**Figure 6**), first proposed in the control of cerebral blood flow [20], to explain how LPS contributes to pneumonemia and ALI/ARDS. In this model, capillary endothelial cells, arteriolar endothelial

cells, and smooth muscle cells expressed Kir2.1 channels on cell membranes. There are gap junctions between two endothelial cells and between arteriolar endothelial cells and smooth muscle cells. Cell membrane potential can be transmitted from endothelial cells to adjacent smooth muscle cells via gap junctions. Hence, LPS from blood may increase expression of Kir2.1 on capillary endothelial cell membranes to mediate K^+ influx and induce hyperpolarization. This hyperpolarization may be transmitted to adjacent arteriolar endothelial cells and smooth muscle cells via gap junction, which can close voltage-dependent-Ca^{2+} channels as well as large conductance Ca^{2+}-activated K^+ channels and voltage-gated K^+ channels, reduce Ca^{2+} influx, and cause smooth muscle relaxation and further pulmonary vasodilation. Therefore, relaxed pulmonary arteries may increase blood flow in the pulmonary vessel bed, leading to pulmonary congestion contributing to pneumonemia and ALI/ARDS.

ALI/ARDS are complex syndromes characterized by pulmonary edema and excessive inflammatory responses induced by LPS. Kir2.1 channel is associated with hyperpolarization and vasodilation and is expressed widely in microvascular endothelial cells from head to toe. Kir2.1 channels likely play a role in conducting vasodilation signaling from endothelial cells on capillary beds to smooth muscle layers. In our study, we found that LPS treatment induced increasing expression and current of Kir2.1 in mouse pulmonary microvascular endothelial cells. Based on previous knowledge about Kir2.1 in vessels, we proposed a model to explain how LPS induces pneumonemia and ALI/ARDS.

Acknowledgements

This work was supported by grants from the National Natural Science Foundation of China (Grant numbers 81701879, 81570403, 81600286); Anhui Provincial Natural Science Foundation (Grant number 1508085QH164); Overseas Scholars Start Fund from Department of Human Resources and Social Security of Anhui Province; Outstanding Young Investigator of Anhui Medical University.

Disclosure of conflict of interest

None.

Address correspondence to: Dr. Bing Shen, Department of Physiology, Anhui Medical University, 81 Meishan Road, Hefei 230032, China. Tel: +86-551-65161132; Fax: +86-551-65161126; E-mail: shenbing@ahmu.edu.cn; Wei Fang, Department of ICU, The Affiliated Hospital of Qingdao University, 16 Jiangsu Road, Qingdao 266071, China. Tel: +86-532-82912326; E-mail: doctorfang@163.com

References

[1] Ware LB and Matthay MA. The acute respiratory distress syndrome. N Engl J Med 2000; 342: 1334-1349.

[2] Rubenfeld GD, Caldwell E, Peabody E, Weaver J, Martin DP, Neff M, Stern EJ and Hudson LD. Incidence and outcomes of acute lung injury. N Engl J Med 2005; 353: 1685-1693.

[3] Mirzapoiazova T, Kolosova IA, Moreno L, Sammani S, Garcia JG and Verin AD. Suppression of endotoxin-induced inflammation by taxol. Eur Respir J 2007; 30: 429-435.

[4] Chen H, Bai C and Wang X. The value of the lipopolysaccharide-induced acute lung injury model in respiratory medicine. Expert Rev Respir Med 2010; 4: 773-783.

[5] Tomashefski JF Jr. Pulmonary pathology of acute respiratory distress syndrome. Clin Chest Med 2000; 21: 435-466.

[6] Pugin J, Verghese G, Widmer MC and Matthay MA. The alveolar space is the site of intense inflammatory and profibrotic reactions in the early phase of acute respiratory distress syndrome. Crit Care Med 1999; 27: 304-312.

[7] Cheng DS, Han W, Chen SM, Sherrill TP, Chont M, Park GY, Sheller JR, Polosukhin VV, Christman JW, Yull FE and Blackwell TS. Airway epithelium controls lung inflammation and injury through the NF-kappa B pathway. J Immunol 2007; 178: 6504-6513.

[8] Kim HJ, Lee HS, Chong YH and Kang JL. p38 Mitogen-activated protein kinase up-regulates LPS-induced NF-kappaB activation in the development of lung injury and RAW 264.7 macrophages. Toxicology 2006; 225: 36-47.

[9] Hibino H, Inanobe A, Furutani K, Murakami S, Findlay I and Kurachi Y. Inwardly rectifying potassium channels: their structure, function, and physiological roles. Physiol Rev 2010; 90: 291-366.

[10] Nichols CG and Lopatin AN. Inward rectifier potassium channels. Annu Rev Physiol 1997; 59: 171-191.

[11] Nehring RB, Wischmeyer E, Doring F, Veh RW, Sheng M and Karschin A. Neuronal inwardly rectifying K(+) channels differentially couple to PDZ proteins of the PSD-95/SAP90 family. J Neurosci 2000; 20: 156-162.

[12] Jackson WF. Potassium channels in the peripheral microcirculation. Microcirculation 2005; 12: 113-127.
[13] Burns WR, Cohen KD and Jackson WF. K+-induced dilation of hamster cremasteric arterioles involves both the Na+/K+-ATPase and inward-rectifier K+ channels. Microcirculation 2004; 11: 279-293.
[14] Hoger JH, Ilyin VI, Forsyth S and Hoger A. Shear stress regulates the endothelial Kir2.1 ion channel. Proc Natl Acad Sci U S A 2002; 99: 7780-7785.
[15] Lieu DK, Pappone PA and Barakat AI. Differential membrane potential and ion current responses to different types of shear stress in vascular endothelial cells. Am J Physiol Cell Physiol 2004; 286: C1367-1375.
[16] Yamamura H, Suzuki Y, Yamamura H, Asai K and Imaizumi Y. Hypoxic stress up-regulates Kir2.1 expression and facilitates cell proliferation in brain capillary endothelial cells. Biochem Biophys Res Commun 2016; 476: 386-392.
[17] Salomonsson M, Brasen JC and Sorensen CM. Role of renal vascular potassium channels in physiology and pathophysiology. Acta Physiol (Oxf) 2017; 14-31.
[18] Zhao R, Zhou M, Li J, Wang X, Su K, Hu J, Ye Y, Zhu J, Zhang G, Wang K, Du J, Wang L and Shen B. Increased TRPP2 expression in vascular smooth muscle cells from high-salt intake hypertensive rats: the crucial role in vascular dysfunction. Mol Nutr Food Res 2015; 59: 365-372.
[19] Lau OC, Shen B, Wong CO, Tjong YW, Lo CY, Wang HC, Huang Y, Yung WH, Chen YC, Fung ML, Rudd JA and Yao X. TRPC5 channels participate in pressure-sensing in aortic baroreceptors. Nat Commun 2016; 7: 11947.
[20] Longden TA and Nelson MT. Vascular inward rectifier K+ channels as external K+ sensors in the control of cerebral blood flow. Microcirculation 2015; 22: 183-196.
[21] Barry DM, Trimmer JS, Merlie JP and Nerbonne JM. Differential expression of voltage-gated K+ channel subunits in adult rat heart. Relation to functional K+ channels? Circ Res 1995; 77: 361-369.
[22] Takahashi T. Inward rectification in neonatal rat spinal motoneurones. J Physiol 1990; 423: 47-62.
[23] Adams DJ and Hill MA. Potassium channels and membrane potential in the modulation of intracellular calcium in vascular endothelial cells. J Cardiovasc Electrophysiol 2004; 15: 598-610.
[24] Jackson WF. Boosting the signal: endothelial inward rectifier K+ channels. Microcirculation 2017; 24: e12319.
[25] de Wit C and Griffith TM. Connexins and gap junctions in the EDHF phenomenon and conducted vasomotor responses. Pflugers Arch 2010; 459: 897-914.
[26] Nilius B and Droogmans G. Ion channels and their functional role in vascular endothelium. Physiol Rev 2001; 81: 1415-1459.
[27] Bradley KK, Jaggar JH, Bonev AD, Heppner TJ, Flynn ER, Nelson MT and Horowitz B. Kir2.1 encodes the inward rectifier potassium channel in rat arterial smooth muscle cells. J Physiol 1999; 515: 639-651.
[28] Nilius B, Schwarz G and Droogmans G. Modulation by histamine of an inwardly rectifying potassium channel in human endothelial cells. J Physiol 1993; 472: 359-371.
[29] Kwan HY, Leung PC, Huang Y and Yao X. Depletion of intracellular Ca2+ stores sensitizes the flow-induced Ca2+ influx in rat endothelial cells. Circ Res 2003; 92: 286-292.
[30] Wellman GC and Bevan JA. Barium inhibits the endothelium-dependent component of flow but not acetylcholine-induced relaxation in isolated rabbit cerebral arteries. J Pharmacol Exp Ther 1995; 274: 47-53.
[31] Knot HJ, Zimmermann PA and Nelson MT. Extracellular K(+)-induced hyperpolarizations and dilatations of rat coronary and cerebral arteries involve inward rectifier K(+) channels. J Physiol 1996; 492: 419-430.