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Disruption of IDH2 attenuates lipopolysaccharide-induced inflammation and lung injury in an α-ketoglutarate-dependent manner

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ABSTRACT

Acute lung injury (ALI) is an acute failure of the respiratory system with unacceptably high mortality, for which effective treatment is urgently necessary. Infiltrations by immune cells, such as leukocytes and macrophages, are responsible for the inflammatory response in ALI, which is characterized by excessive production of pro-inflammatory mediators in lung tissues exposed to various pathogen-associated molecules such as lipopolysaccharide (LPS) from microbial organisms. α-Ketoglutarate (α-KG) is a key metabolic intermediate and acts as a pro-inflammatory metabolite, which is responsible for LPS-induced proinflammatory cytokine production through NF-κB signaling pathway. Mitochondrial $NADP^+$-dependent isocitrate dehydrogenase (IDH2) has been reported as an essential enzyme catalyzing the conversion of isocitrate to α-KG with concurrent production of NAPDH. Therefore, we evaluated the role of IDH2 in LPS-induced ALI using IDH2-deficient mice. We observed that LPS-induced inflammation and lung injury is attenuated in IDH2-deficient mice, leading to a lengthened life span of the mice. Our results also suggest that IDH2 disruption suppresses LPS-induced proinflammatory cytokine production, resulting from an inhibition of the NF-κB signaling axis in an α-KG-dependent manner. In conclusion, disruption of IDH2 leads to a decrease in α-KG levels, and the activation of NF-κB in response to LPS is attenuated by reduction of α-KG levels, which eventually reduces the inflammatory response in the lung during LPS-induced ALI. The present study supports the rationale for targeting IDH2 as an important therapeutic strategy for the treatment of systemic inflammatory response syndromes, particularly ALI.

1. Introduction

Acute lung injury (ALI) has been recognized as a life-threatening disease with clinical symptoms such as bilateral pulmonary infiltration, which increases vascular permeability and arouses pulmonary edema [1,2]. It is a common clinical problem that presents high morbidity and mortality rates worldwide [3,4]. ALI often results from sepsis, aspiration, or blood transfusion, and presents a high mortality rate of 30–50% [5,6]. Sepsis, a major cause of the high mortality rate of ALI, occurs in patients with gram-negative bacterial infections [7].

Lipopolysaccharide (LPS) is widely accepted to establish sepsis-induced ALI models [8]. LPS is an endotoxin derived from the outer membranes of gram-negative bacteria and is the main trigger for the innate immune and acute inflammatory responses that are vital for antimicrobial defense reactions [9]. LPS binds Toll-like receptor 4 (TLR4) to activate a crucial proinflammatory transcription factor NF-κB that elicits the expression and release of numerous inflammatory mediators, including TNF-α, IL-1β, and IL-6 [10–12].

α-Ketoglutarate (α-KG) is considered a pro-inflammatory metabolite that increases the LPS-induced production of cytokines such as IL-1β and TNF-α, the levels of which, in particular, affect HIF-1 activity, a key factor in the regulation of the NF-κB signaling axis in an IKK-dependent manner [13]. The mitochondrial $NADP^+$-dependent isocitrate dehydrogenase (IDH2) catalyzes the conversion of isocitrate to α-KG with concurrent production of NAPDH in mitochondria [14]. Therefore, IDH2 is implicated as a

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critical factor in the regulation of the NF-κB-mediated proinflammatory effect in response to LPS. However, there is no evidence showing a potential effect of IDH2 in LPS-induced ALI. In the present study, we used IDH2-deficient mice to investigate the functional relationship between IDH2 and lung injury in the LPS-induced ALI model.

2. Materials and methods

2.1. Animals and study design

All animal experiments were reviewed and approved by the Kyungpook National University Institutional Animal Care and Use Committee. Experiments were performed using 8-week-old male C57BL/6 mice with different genotypes, including wild-type $idh2^{+/+}$ (WT) and knockout $idh2^{-/-}$ (KO) mice generated by breeding and identified by PCR genotyping, as previously described [15]. The mice were housed in microisolator rodent cages at 22 °C with a 12 h light/dark cycle and allowed free access to water and standard mouse chow. Mice were divided into four groups, with 6–10 mice per group (WT, WT + LPS, KO, and KO + LPS). To evaluate the effect of IDH2 on the outcome of LPS-induced ALI, a 5 mg/kg dose of LPS dissolved in saline (*Escherichia coli* O55:B5; Sigma Aldrich) was injected into the mouse peritoneal cavity.

2.2. Morphological assessments

For morphological assessments, lung tissues were harvested from the mice after LPS treatment, and then fixed with 4% formalin. Five micrometer paraffin lung sections were stained sequentially with hematoxylin and eosin (H&E). First, in order to determine the air space enlargement in the lungs [16], air space areas (alveoli diameter) were measured and quantified using the ImageJ software (Bethesda, MD, USA). Afterwards, lung injury was graded from 0 (normal) to 4 (severe) in four categories: interstitial inflammation, neutrophil infiltration, congestion, and edema [17]. Similarly, the severity of lung inflammation were graded on a scale of 0–8 [18] and 0–3 [19], respectively. Lung injury severity was calculated by adding the individual scores for each category and all histology analyses were performed by three pathologists in a blinded fashion.

2.3. Immunohistochemical analysis

Lungs were harvested and fixed with 4% formalin in PBS and embedded in paraffin. Then, 5 μm thick tissue sections were deparaffinized, rehydrated, and used for immunohistochemistry and immunofluorescence. For antigen retrieval, the slides were submerged in 10 mM sodium citrate (pH 6.0) and heated to 90 °C for 20 min. The slides were rinsed in 50 mM Tris-HCl with 150 mM NaCl at pH 7.5 and blocked with 3% BSA in TBS with 0.05% Tween 20. Immunohistochemistry was performed using a VECTASTAIN ABC Kit (PK-4001, Vector Laboratories, Burlingame, CA, USA), according to the manufacturer's instructions. Images were acquired with a light microscope (Nikon Eclipse 80i) or a confocal microscope (Nikon E600).

2.4. CT scan analysis

After mice were anesthetized with isoflurane, mouse lung imaging was performed with the small animal Inveon system (Siemens Medical Solutions, USA). Briefly, the micro CT scanner was set to 80 kVp for the X-ray tube and 500 μA for the X-ray source, with an exposure time of 250 m s. The detector and X-ray sources were rotated 360° with 180 rotation steps, 30 calibration exposures, and the field of view (FOV) was set at 56.89 × 56.89 mm. CT reconstruction was used to standardize the cone-beam setup with

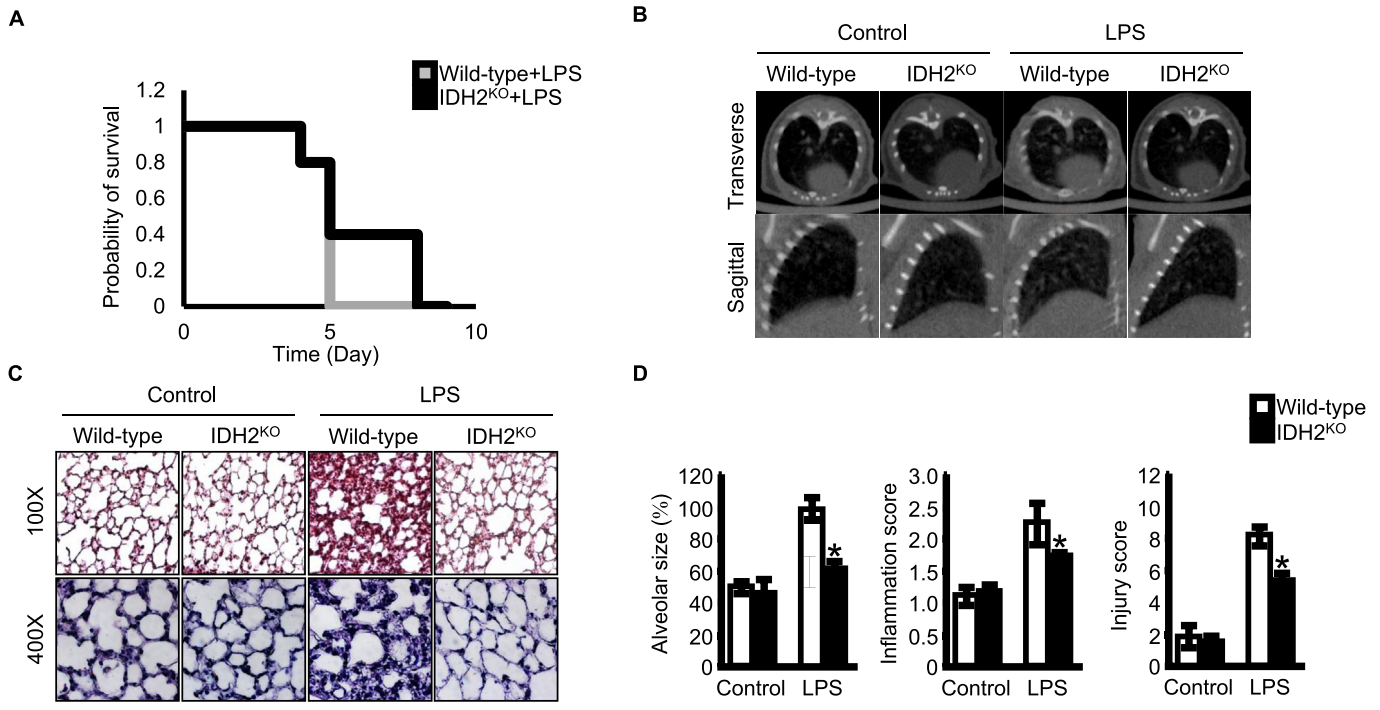

Fig. 1. Effect of IDH2 deficiency on LPS-induced ling injury. (A) Kaplan-Meier survival analysis of WT and IDH2-KO male mice exposed to LPS. (B) Lung cavity and representative cross-sectional CT images of wild-type and IDH2-KO mice. (C) Lung pathology and morphometric assessment of pulmonary injuries (alveolar size, inflammation score, and injury score). H&E staining was carried out to exam the lung sections from wild-type and IDH2-KO mice. Results are shown as mean ± SD (n = 4–6 in each group). *$p < 0.05$ versus LPS-treated WT mice.

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