ORIGINAL ARTICLE

Parkin regulates lipopolysaccharide-induced proinflammatory responses in acute lung injury

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The acute respiratory distress syndrome (ARDS) is a serious condition resulting from direct or indirect lung injury that is associated with high mortality and morbidity. A key biological event in the pathogenesis of the acute lung injury (ALI) that causes acute respiratory distress syndrome is activation of the lung endothelium cells (ECs), which is triggered by a variety of inflammatory insults leading to barrier disruption and excessive adhesion/activation of neutrophils. Recently, we demonstrated that imatinib protects against lipopolysaccharide (LPS)-induced EC activation by inhibiting c-Abl kinase. In the present study, we explored the role of parkin, a novel c-Abl substrate, in ALI. Parkin is an E3 ubiquitin ligase originally characterized in the pathogenesis of Parkinson disease; however, its potential role in acute inflammatory processes and lung EC function remains largely unknown. Using parkin deficient (PARK2−/−) mice, we now demonstrate a crucial role for parkin in mediating LPS-induced ALI. After LPS, PARK2−/− mice have reduced total protein and cell levels in bronchoalveolar lavage compared to wild type. Moreover, in LPS-treated PARK2−/− lungs, the sequestration/activation of neutrophils and release of inflammatory cytokines (IL-6, TNF-α) are significantly reduced. The bronchoalveolar lavage levels of soluble VCAM-1 and ICAM-1 are also decreased in LPS-treated PARK2−/− mice compared to wild type. In cultured human lung endothelial cells, downregulation of parkin by small interfering RNA decreases LPS-induced VCAM-1 expression, IL-8 and IL-6 secretion, and NF-kB phosphorylation. These results suggest a previously unidentified role of parkin in mediating endotoxin-induced endothelial proinflammatory signaling and indicate that it may play a critical role in acute inflammation. (Translational Research 2016;■:1–12)

Abbreviations: ARDS = acute respiratory distress syndrome; ALI = acute lung injury; ICU = intensive care unit; LPS = lipopolysaccharide; EC = endothelial cells; HPAECs = human pulmonary artery endothelial cells; NF-kB = nuclear factor kappa-light-chain-enhancer of activated B cells; PARK2 = parkin deficient; ICAM-1 = intracellular adhesion molecule-1; VCAM-1 = vascular cell adhesion molecule 1; siRNA = small interfering RNA

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AT A GLANCE COMMENTARY

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Background

This study investigates the role of parkin, an E3 ubiquitin ligase, in regulating lung inflammation and endothelial dysfunction in a preclinical model of acute lung injury/acute respiratory distress syndrome.

Translational Significance

Our study demonstrates that parkin is functionally involved in acute lung injury pathogenesis and mediates lung endothelial activation and proinflammatory mediator production in response to lipopolysaccharide. These findings highlight a new role for parkin in the regulation of acute inflammation and also add novel insights into the biology of parkin, which is involved in the pathogenesis of several diseases and may represent a therapeutic target for future studies.

INTRODUCTION

Acute lung injury (ALI) processes such as the acute respiratory distress syndrome (ARDS) are life-threatening lung diseases characterized by an excessive lung inflammatory response and increased alveolo-capillary permeability leading to edema, hypoxemia, and respiratory failure.[1] Recent epidemiologic studies demonstrate that ARDS remains an important public health problem globally[2] and a major challenge for clinicians; it affects an estimated 200,000 people annually in the US[3] and 2.2 million worldwide, and is associated with high in-hospital mortality (~40%) despite advances in critical care.[2] There are several medical conditions that can cause ARDS, such as lung infections, sepsis, and serious trauma, but currently, there is no Food and Drug Administration–approved effective pharmacologic treatment.[1] Moreover, there are substantial gaps in our knowledge about the underlying mechanisms of the pathogenesis of ALI/ARDS that hinder development of new therapies.

A key event in the pathogenesis of ALI/ARDS is dysfunction of the lung endothelial cell (EC), which is triggered by a variety of inflammatory insults leading to damaged EC barrier, vascular leak, and sustained inflammation.[4,5] Recent work by our group and others has identified a novel role for c-Abl nonreceptor tyrosine kinase to mediate EC dysfunction and activation induced by ALI-relevant stimuli.[6-9] We recently demonstrated that imatinib, a Food and Drug Administration–approved Abl kinase inhibitor, exhibits pulmonary barrier protective and antiinflammatory effects in lipopolysaccharide (LPS)-injured mice (pre- and post-treatment) and in a 2-hit (LPS plus high-tidal volume mechanical ventilation) murine ALI model.[6,10] Other recent studies have also demonstrated the protective actions of imatinib against additional experimental models of ALI[11-13] and provide further evidence to support the hypothesis that blocking c-Abl signaling may prevent or ameliorate lung injury. On activation, c-Abl kinase subsequently phosphorylates a variety of substrates, which mediate its downstream effects, such as CRK-like protein (CRKL),[14] cytoskeletal effector proteins (eg, nmMLCK, cortactin),[15] VAV1,[16] dynamin 2,[17] and parkin, an E3 ubiquitin ligase.[18,19]

Parkin has been studied primarily as a mitophagy regulator in the context of Parkinson disease because mutations in its gene (PARK2) are associated with development of that neurologic disorder.[20,21] More recent studies suggest Q6 an important role for this protein in multiple other diseases such as pulmonary emphysema and COPD, pulmonary fibrosis, and sepsis.[22-24] However, the potential functional role of parkin in ALI/ARDS and whether it regulates lung endothelial function under acute inflammatory conditions are completely unknown, and this study focuses on exploring this important knowledge gap. Our data not only demonstrate that parkin is involved in the pathogenesis of ALI but also provide strong evidence that it mediates proinflammatory responses in lung EC similar to c-Abl.[6]

MATERIALS AND METHODS

Animal experiments. All procedures were approved by the Animal Care Committee of the University of Illinois at Chicago (UIC). C57BL/6 wild-type (WT) mice and PARK2−/− mice on C57BL/6 background, obtained from Jackson Laboratory (Bar Harbor, Maine), were bred and maintained in autoclaved cages with free access to food and water in the UIC facilities. Male 10- to 12-week-old mice were used for all experiments. PARK2−/− and WT mice were challenged with LPS Q7 (1 mg/kg) or PBS (control group) intratracheally and after 18 hours, bronchoalveolar lavage (BAL) fluid and lungs were harvested and processed, as we have described.[6] Briefly, BAL was centrifuged at 500 × *g* for 20 minutes to pellet the BAL cells. Red blood cells were lysed using red blood cell lysis buffer (Qiagen, Valencia, Calif), and total BAL cell counts were measured using the Bio-Rad TC10 automated cell counter device. Differential counting of BAL cells was performed by microscopy of cytospin preparations after staining with Kwik-Diff Stain (Thermo Scientific,