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Omentin-1 protects against bleomycin-induced acute lung injury

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ABSTRACT

Acute lung injury (ALI) is characterized by inflammatory cell infiltration, macrophage activation, and excessive pro-inflammatory cytokine production. Bleomycin (BLM) is widely used to induce acute lung injury (ALI) and fibrosis in murine models. Intratracheally administration of BLM leads to the early stage of inflammatory response and the late stage of collagen deposition. Omentin-1 exerts an anti-inflammatory role in reducing tumor necrosis factor α (TNF-α)-induced cyclooxygenase-2 expression in endothelial cells and attenuating lipopolysaccharide (LPS)-induced ALI. However, the role of omentin-1 in BLM-induced ALI remains unclear. The aim of this study is to examine the effects of omentin-1 on BLM-induced ALI. We found that omentin-1 was decreased in lungs of BLM-induced ALI mice. Omentin-1 overexpression mediated by adenovirus alleviated lung injury and maintained the integrity of the alveolar septa. Omentin-1 overexpression also remarkably decreased the aggregation of neutrophil and macrophages activation, the expression of monocyte chemotactic protein 1 (MCP-1), and down-regulated expression of interleukin 1β (IL-1β) in lungs of BLM-induced ALI mice. Furthermore, we observed that omentin-1 reduced oxidative stress and suppressed the activation of NF-κB pathway in BLM-induced ALI and LPS-induced macrophages activation. Together, our findings indicated that omentin-1 protected mice from BLM-induced ALI may through reducing inflammatory cells recruitment and macrophages activation *via* alleviation of oxidative stress and NF-κB pathway. Thus, therapeutic strategies aiming to restore omentin-1 levels may be valuable for the prevention of BLM-induced ALI.

1. Introduction

Acute lung injury (ALI) is a debilitating condition, which is characterized by inflammatory cell infiltration, macrophage activation, and excessive pro-inflammatory cytokine production (Sun et al., 2018; Ying et al., 2015). Bleomycin (BLM), a chemotherapeutic drug used in the treatment of various human malignant tumors, can induce lung injury and pulmonary fibrosis at high doses (Gouda et al., 2018). Therefore, it is widely used to induce ALI and fibrosis in animal models (Birjandi et al., 2016; Steffen et al., 2017). Intratracheal administration of BLM causes acute lung inflammation during the first week and pulmonary fibrosis in the second and third weeks after administration (Liu et al., 2013). The acute inflammatory response plays a major role in the development of pulmonary fibrosis induced by BLM. Treatment with anti-inflammatory drugs in the first three days after BLM challenge prevents the development of BLM-induced fibrosis (Liu et al., 2013). The pathological alterations induced by BLM include injuries to alveolar epithelial cells and vascular endothelial cells, infiltration of alveolar inflammatory cell, activation of macrophages, and increase in pro-inflammatory cytokine levels (Tian et al., 2018). Furthermore, oxidative stress and toxic reactive oxygen species (ROS) production play an important role in the accumulation and activation of inflammatory cells

Abbreviation: ALI, acute lung injury; BLM, bleomycin; TNF-α, tumor necrosis factor α; LPS, lipopolysaccharide; MCP-1, monocyte chemotactic protein 1; IL-1β, interleukin 1β; NF-κB, nuclear factor-κB; ROS, reactive oxygen species; Ad-omentin-1, adenovirus omentin-1; Ad-β-gal, adenovirus β-galactosidase; BALF, bronchoalveolar lavage fluid; H&E, hematoxylin and eosin; ELISA, enzyme-linked immunosorbnent assay; qPCR, quantitative polymerase chain reaction; MDA, malondialdehyde; SOD, superoxide dismutase; mtROS, mitochondrial ROS; IκB, inhibitor of κB

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in the lungs, leading to lung injury and lung fibrosis (Amirshahrokhi and Khalili, 2016; Gonzalez-Gonzalez et al., 2017).

Inflammatory cells infiltration, macrophage activation and excessive pro-inflammatory cytokine production were responsible for the inflammatory responses in the lungs (Kapoor et al., 2015; Zhou et al., 2017). Macrophages, which act as the first line of defense in the lungs, play an important role in the initiation, propagation, and resolution of inflammation. In terms of the inflammatory response, Macrophages polarization is a phenotypical change for responding to variations in the microenvironment. Polarized macrophages are classified into two main subsets: classically activated (M1) and alternatively activated (M2) macrophages. M1 macrophages are termed as pro-inflammatory macrophages which secrete TNF-α, and IL-1β to initiate the recruitment of inflammatory cells. The macrophages that inhibit inflammatory response and enhance tissue repair are termed as M2 macrophages (Wang et al., 2018). The balance in M1/M2 is significant to regulate inflammatory response. Therefore, it is extremely important to develop an effective strategy to regulate M1/M2 macrophage activation, which are of great significance for ALI treatment.

Omentin-1 is a novel hydrophilic adipokine of 313 amino acids (35 kDa), which contains a secretory signal sequence and a fibrinogen-related domain, and appears as a glycolized trimer of 120 kDa molecular weight in its negative form (Fain et al., 2008; Yang et al., 2006). It was initially identified in intestinal Paneth cells and endothelial cells with the name intelectin-1, intestinal lactoferrin receptor, galactofuranose binding lectin, and endothelial lectin (Kapoor et al., 2015; Tan et al., 2010). However, specific receptors for omentin-1 have not yet been identified (Tan et al., 2015; Zhou et al., 2018). Omentin-1 is an anti-inflammatory adipokine that is abundant in human visceral fat tissue (Tan et al., 2010). Experimental studies have found that omentin-1 stimulated vasodilation in isolated blood vessels and suppressed cytokine-stimulated inflammation in endothelial cells (Yamawaki et al., 2011, 2010). Decreased circulating omentin-1 levels could be regarded as an independent predictive marker for obstructive sleep apnea syndrome (Zhou et al., 2018); omentin-1 protected against pulmonary arterial hypertension by suppressing vascular structure remodeling and abnormal contractile reactivity (Kazama et al., 2014). Although omentin-1 has been reported to play an important protection role in LPS-induced ALI through suppressing pulmonary inflammation and promoting endothelial barrier *via* an Akt/eNOS-dependent mechanism (Qi et al., 2016). However, its role in BLM-induced ALI remains unclear.

In the current study, we aimed to appraise the significance of omentin-1 in BLM-induced ALI and specifically evaluated its impact on accumulation of inflammatory cells and activation of macrophage and the possible intercellular mechanism in murine macrophages.

2. Materials and methods

2.1. Ethics statement

The Ethics Committee of the Center for Scientific Research with Animal Models at Central South University (Changsha, China) approved the experiments in this study, which were performed in accordance with the guidelines of the National Institutes of Health. The mice were anesthetized with chloral hydrate (400 mg/kg, intraperitoneal injection), and all efforts were made to minimize suffering before performing the operations.

2.2. Animal models

Animal experiments were conducted on 8-week-old female C57BL/6 mice (specific-pathogen-free [SPF] grade; Department of laboratory animal unit of Central South University). The mice were housed under pathogen-free conditions with a 12 h dark/light cycle and provided food and water *ad libitum*. All animal experimental protocols were implemented according to the instructions of the National Institutes of Health Guide for the Care and Use of Laboratory Animals. The adenovirus producing full-length mouse omentin-1 was prepared using the Adenovirus Expression Vector Kit (Takara, Japan). Adenovirus omentin-1 (Ad-omentin-1) or adenovirus β-galactosidase (Ad-β-gal; control) used at 5×10^7 PFU per mouse was injected into the tail vein of mice 3 days before BLM or vehicle (phosphate-buffered saline [PBS]) airway installation. On the third day after adenovirus injection (Day 0), the mice were intratracheally injected with 50 μl BLM (3.5 mg kg-1) or sterile PBS after being anesthetized. Lung samples and bronchoalveolar lavage fluid (BALF) were collected on Day 3 after BLM administration for further experiments or stored at −80 °C until further analysis.

2.3. Hematoxylin and Eosin (H&E) staining and lung histology evaluation

The upper right lobe of the lungs was isolated, fixed in 4% paraformaldehyde, embedded in paraffin wax, cut into 5-μm-thick sections, and stained with hematoxylin and eosin (H&E). Histological lung injury in each mouse was evaluated in five random fields. The lung injury score was measured by two blinded pathologists. Five independent variables were evaluated using a 0–4 point scale (Duan et al., 2017): neutrophils in the alveolar space, hemorrhage, hyaline membranes, pertinacious debris filling the airspaces, and septal thickening. A score of 0 represented no damage; l, mild damage; 2, moderate damage; 3, severe damage; and 4, very severe histological changes.

2.4. Analysis of BALF

The mice were anesthetized, and after the trachea was exposed, a plastic cannula was inserted into the trachea. A syringe was connected to the catheter, and 0.8 ml 0.9% saline solution was infused into the airway, following which aspiration was performed three times. BALF samples were centrifuged at 500 × *g* for 10 min at 4 °C. The pellets were resuspended in 50 μl PBS. Total cell counts were performed with a hemocytometer in a double-blind manner. The protein levels in the BALF supernatants were determined using a bicinchoninic acid protein assay (BCA) kit (Thermo Fisher Scientific, USA) according to the manufacturer's instructions.

2.5. Enzyme-linked immunosorbent Assay (ELISA) for cytokine measurements

ELISA was used to determine the omentin-1 levels in the lung homogenate and the MCP-1 levels in the lung homogenate and BALF. After thoracotomy, the lungs were removed and homogenized in PBS containing protease inhibitors (Thermo Fisher Scientific, USA). The lung homogenates were centrifuged at 10,000 × *g* to remove insoluble debris. The supernatants of lung homogenates were assayed with anti-mouse omentin-1 ELISA kits (CUSABIO, China). The supernatants of lung homogenates and BALF were assayed with anti-mouse MCP-1 ELISA kits according to the manufacturer's instructions (Invitrogen, USA). For *in vitro* analysis, the supernatants of the cells were used for tumor necrosis factor α (TNF-α) and interleukin 1 β (IL-1β) analysis with a commercially available anti-mouse IL-1β and anti-mouse TNF-α ELISA kit (Invitrogen, USA).

2.6. Primary peritoneal macrophage culture

The 8-week-old female C57BL/6 mice were injected (intraperitoneal injection) with 3 ml 3% thioglycolate (Sigma-Aldrich, USA). After 3 days, peritoneal macrophages were harvested by peritoneal lavage with precooled RPMI 1640 (Hyclone, USA). The cells were collected by centrifugation at 1500 × *g* for 10 min at 4 °C and resuspended in cell culture medium. They were plated in 12-well plates at a density of 1×10^6 cells/well; 2 h later, the culture medium was changed completely to remove the nonadherent cells. Macrophages were cultured in a humified CO_2 incubator at 37 °C and rested for 24 h before subsequent