Experimental Gerontology 57 (2014) 29–40

Contents lists available at ScienceDirect

Experimental Gerontology

journal homepage: www.elsevier.com/locate/expgero

Age-related activation of MKK/p38/NF-κB signaling pathway in lung: From mouse to human

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ARTICLE INFO

Article history:
Received 22 December 2013
Received in revised form 27 April 2014
Accepted 29 April 2014
Available online 5 May 2014

Section Editor: B. Grubeck-Loebenstein

Keywords:
P38
Lung
Inflammaging
Lipopolysaccharide
Cytokines

ABSTRACT

We and others previously reported that the pro-inflammatory cytokine tumor necrosis factor-α (TNF-α), interleukin-1β (IL-1β) and IL-6 significantly accumulate with age in mouse lung. This is accompanied by elevated phosphorylation of p38. Here, we further investigate whether aging affects activation of p38 signaling and the inflammatory reaction after exposure to lipopolysaccharide (LPS) in the lungs of mice in vivo and humans ex vivo. The data showed that activation of p38 peaked at 0.5 h and then rapidly declined in young (2-month-old) mouse lung, after intranasal inhalation challenge with LPS. In contract, activation of p38 peaked at 24 h and was sustained longer in aged (20-month-old) mice. As well as altered p38, activations of its upstream activator MKK and downstream substrate NF-κB were also changed in the lungs of aged mice, which corresponded with the absence in the early phase but delayed increases in concentrations of TNF-α, IL-1β and IL-6. Consistent with the above observations in mice, similar patterns of p38 signaling also occurred in human lungs. Compared with younger lungs from adult–middle aged subjects, the activation of p38, MKK and NF-κB, as well as the production of pro-inflammatory cytokines were significantly increased in the lungs of older subjects ex vivo. Exposure of human lung cells to LPS induced rapid activation of p38, MKK and NF-κB in these cells from adult–middle aged subjects, but not older subjects, with increases in the production of the pro-inflammatory cytokines. The LPS-induced rapid activation in the lung cells from adult–middle aged subjects occurred as early as 0.25 h after exposure, and then declined. Compared with adult–middle aged subjects, the LPS exposure did not induce marked changes in the early phase, either in the activation of p38, MKK and NF-κB, or in the production of TNF-α, IL-1β or IL-6 in the lung cells from older subjects. In contrast, these changes occurred relatively late, peaked at 16 h and were sustained longer in the lungs of older subjects. These data support the hypothesis that the sustained activation of the p38 signaling pathway at baseline and the absence in the early phase but delayed of p38 signaling pathway response to LPS in the elderly may play important roles in increased susceptibility of aged lungs to inflammatory injury.

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Abbreviations: ALI, acute lung injury; ANOVA, analysis of variance; BALF, bronchoalveolar lavage fluid; COPD, chronic obstructive pulmonary disease; DAB, 3, 3′-diaminobenzidine tetrahydrochloride; DAPI, 4′, 6-diamidino-2-phenylindole; DMSO, dimethyl sulphoxide; ERKs, extracellular signal-regulated kinases; FBS, fetal bovine serum; FEV_1, forced expiratory volume in 1 s; FVC, forced vital capacity; H&E, Hematoxylin and eosin; IL-1β, interleukin-1β; IL-6, interleukin-6; i.n, intranasal inhalation; INF-γ, interferon-γ; JNKs, c-Jun N-terminal kinases; LPS, lipopolysaccharide; MAPKs, mitogen activated protein kinases; MKK, mitogen activated protein kinase kinase; MKPs, MAPK phosphatases; NF-κB, nuclear factor-kappa B; OCT, optimum cutting temperature compound; PBS, phosphate-buffered saline; PLSD, protected least significant difference; P-MKK, phosphorylated-MKK; P-p38, phosphorylated-p38; PVDF, polyvinylidene floride; SA β-gal, senescence-associated β-galactosidase; SAPKs, stress-activated protein kinases; SEM, standard error of mean; TLR, Toll-like receptor; TNF-α, tumor necrosis factor-α; W/D, wet/dry.

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http://dx.doi.org/10.1016/j.exger.2014.04.017
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1. Introduction

Inflammaging is an emerging concept based on a progressive increase in pro-inflammatory status during the aging process (Franceschi et al., 2000). Some studies have shown that pulmonary inflammation progressively increases with age (Rahman and Adcock, 2006) and that there is an age-dependent increase of interleukin-6 (IL-6), interferon-γ (INF-γ), and tumor necrosis factor-α (TNF-α) in the airways of humans and rodents (Aoshiba and Nagai, 2007; Sharma et al., 2009). We previously also reported that the concentrations of pro-inflammatory cytokines TNF-α, IL-1β and IL-6 were elevated in lung tissues and in bronchoalveolar lavage fluid (BALF) of mice with age (Li et al., 2011). These mediators might contribute to the increased neutrophils observed in the lower respiratory tract of healthy elderly individuals (Nikolaidis et al., 2011; Patel et al., 2012; Pignatti et al., 2011), suggesting that "inflammaging" naturally occurs in the lung with age.

Recent studies indicated that aging can impair lung ability to repair the damaged lung tissues underlying inflammaging (Rahman et al., 2012), leading to aggressive lung destruction when exposed to extrinsic environmental factors. The elderly individuals have increased vulnerability to inflammatory injury, including acute lung injury (ALI) (Meyer, 2010; Rubenfeld et al., 2005), which is characterized by acute inflammation of alveoli resulting in diffuse alveolar injury, recruitment of inflammatory cells including neutrophils to lung parenchyma, release of cytokines, and the disruption of the alveolar-epithelial barrier (Fanelli et al., 2013). Similar observations have also been reported in mice. Exposure to cigarette smoke or bacterial products (such as LPS), or extra stimulations (such as cecal ligation and puncture) potentially promotes a relatively exacerbated pulmonary inflammatory response in aged mice compared with the young controls, and may contribute to pulmonary dysfunction (Gould et al., 2010; Ito et al., 2007; Saito et al., 2003). These studies confirmed that aging may potentially worsen acute lung injury induced by extrinsic environmental factors, although the underlying mechanisms are poorly understood.

P38 signaling pathway plays a key role in stress (Gong et al., 2012). Activated p38 enhances pro-inflammatory responses through modulation of transcription factors, such as NF-κB (Wong et al., 2012), or by altering the stability and translation of the relevant signaling factors at the mRNA level (Buxade et al., 2008; Ronkina et al., 2008). A major role of p38 is to promote the production of some pro-inflammatory cytokines, particularly TNF-α, IL-1β, IL-6 and IL-8 (Chung et al., 2009). Thus, p38 activation in stress response to environmental stimuli, like LPS, may be a key factor in triggering inflammation in the lung. It has been reported that aging affects the activation of p38 signaling in neutrophils when replying to stress, contributing to a pro-inflammatory state in elderly individuals (Chaves et al., 2009; Larbi et al., 2005). Data also showed that there is an alteration with aging in the p38 activation in T cell from the elderly subjects following stimulation (Di et al., 2011; Douziech et al., 2002). Furthermore, we and others previously found that activated p38, but not total p38, was apparently increased in the lungs from aged mice (Li et al., 2011) and elderly individuals (Gaffey et al., 2013). However, it is still unclear whether aging affects the activation of p38 as part of a stress response to an inflammatory stimulation in the lung.

In this study, we attempted to determine whether aging influences the p38 signaling pathway during stress response to LPS in the mouse lung and human lung. Our data demonstrate that aging impairs the p38 signaling pathway in stress response to environment stimuli in the lung, suggesting a novel mechanism by which the lung from the elderly is susceptible to inflammation.

2. Materials and methods

2.1. Animal and subject characteristics

Young (2 months old) and aged (20 months old) male C57BL/6J mice were fed ad libitum with a pelleted stock diet and housed under pathogen-free conditions at Capital Medical University, PR China. The protocol was approved by the Animal Care and Use Committee of Capital Medical University and followed the "principles of laboratory animal care" (NIH Publication No. 86-23, revised 1985).

In addition, a cohort of 11 adult–middle aged subjects (age range, 29–47 years) and 14 older subjects (age range, 62–74 years) with normal lung function undergoing surgical resection for suspected or confirmed lung cancers were recruited from Beijing Chao-Yang Hospital (Table 1). Subjects who had hormone replacement and preexistent conditions such as inflammatory disorders, including connective tissue, tuberculosis and diabetes were excluded. "Normal lung tissue" was collected from an area of the lung as far distal to the tumor as possible, and processed as described previously (Plumb et al., 2009). The study was approved by the Ethics Committee of Beijing Chao-Yang Hospital.

2.2. Administration of LPS and SB203580 in mice

LPS (*Escherichia coli*, serotype O55:B5, Sigma, St. Louis, MO, USA) was dissolved in sterile physiological saline (0.9% NaCl) at a concentration of 200 μg/50 μl. Animals exposed to LPS received a single intranasal (i.n.) inhalation at a dosage of 200 μg. Control animals received only administration of the vehicle control (normal saline). For the time-course of the study, mice received a single i.n. inhalation of 200 μg LPS and were assessed at 0.5, 4, 24 or 72 h later, which were based on the other previous studies (Ito et al., 2005; Maijó et al., 2012; Qiu et al., 2011) and our own preliminary experiments. To test whether targeting p38 inhibits activation of its downstream, NF-κB in aged mice after LPS challenge at 72 h, SB203580 (Biosource International, Camarillo, CA, USA), a specific inhibitor of p38 was given to aged mice (200 μg/50 μl, i.n.) at various time points including 0.5 h prior and 4, 16 and 36 h post LPS inhalation. All the animals were sacrificed and examined at 72 h after LPS inhalation.

2.3. Preparations of BALF and lung tissue homogenate

BALF was performed as previously described (Li et al., 2011). Briefly, lungs dissected from anesthetized animals were lavaged via the trachea with initial 0.8 ml ice-cold isotonic phosphate-buffered saline (PBS, pH 7.2) followed by a consecutive aliquot of PBS (total of 1.5 ml). The two rinses were pooled and centrifuged at 1500 rpm for 10 min at 4 °C, and then processed for cell counts and differentials. The cell-free supernatant was collected and stored at −20 °C for analysis of cytokines.

Lung tissues were harvested after vascular perfusion. To determine the protein levels of MKK, p38 and NF-κB p65, the right cranial lobe of lungs was homogenized at 4 °C in lysis buffer A, B or C. Cytosolic proteins was extracted by Buffer A (50 mM Tris–HCl, pH 7.5, 1 mM dithiothreitol, 2 mM ethylene diamine tetraacetic acid, 2 mM ethylene glycol tetraacetate, 5 mg/ml each of leupeptin, aprotinin, pepstatin A, and chymostatin, 50 mM potassium fluoride, 50 nM okadaic acid, 5 mM sodium pyrophosphate, and 100 μM sodium vanadate). The nuclear pellets were resuspended in buffer B containing all reagents in buffer A and 0.5% NP-40. Buffer C (buffer A and 2% SDS) was used for total cell protein extraction. The protein concentration was determined using a BCA Protein Assay kit (Pierce, Rockford, IL, USA).

2.4. Lung wet/dry weight ratio assessment in mice

The water content of lung tissue was determined by calculating the tissue wet/dry weight ratio. The right middle lobe of lungs was excised, rinsed briefly in PBS, blotted dry and weighed to obtain the "wet" weight. The lung was then immediately dried at 80 °C for 72 h in an oven to obtain the "dry" weight. The ratio of wet lung to dry lung was calculated to assess lung tissue edema (Patel et al., 2012).

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