Free Radical Biology and Medicine 84 (2015) 196–205

Contents lists available at ScienceDirect

Free Radical Biology and Medicine

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

Original Contribution

Adenovirus-mediated transfer of the SOCS-1 gene to mouse lung confers protection against hyperoxic acute lung injury

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ARTICLE INFO

Article history:
Received 31 January 2015
Received in revised form
25 March 2015
Accepted 29 March 2015
Available online 4 April 2015

Keywords:
Adenovirus
Acute lung injury (ALI)
Suppressor of cytokine signaling-1 (SOCS-1)
Hyperoxia
Inflammation
Apoptosis signal-regulating kinase-1 (ASK-1)

ABSTRACT

Suppressor of cytokine signaling-1 (SOCS-1) is a member of the suppressor of cytokine signaling family of proteins and an inhibitor of interleukin-6 (IL-6) signaling. SOCS-1 has been shown to protect cells from cellular damage and apoptosis induced by tumor necrosis factor (TNF), lipopolysaccharide (LPS), and interferon gamma (IL-γ). However, it is not known whether increased SOCS-1 is protective during pulmonary oxidative stress. Therefore, we hypothesized that increased SOCS-1 in the lungs of mice would be protective in the setting of hyperoxic lung injury. We administered SOCS-1 adenovirus (Ad-SOCS-1) intratracheally into the lungs and exposed the mice to 100% O_2. Mice infected with GFP adenovirus (Ad-GFP) were used as controls. Mice treated with Ad-SOCS-1 had enhanced survival in 100% oxygen compared to Ad-GFP-administered mice. After 3 days of hyperoxia, Ad-GFP mice were ill and tachypnic and died after 4 days. In contrast, all Ad-SOCS-1-treated mice survived for at least 6 days in hyperoxia and 80% survived beyond 7 days. Ad-SOCS-1 transfection protected mouse lungs from injury as indicated by lower lung wet/dry weight, alveolar–capillary protein leakage, reduced infiltration of inflammatory cells, and lower content of thiobarbituric acid-reactive substances in lung homogenate. Our results also indicated that Ad-SOCS-1 significantly inhibits hyperoxia-induced ASK-1 (apoptosis signal-regulating kinase 1) expression. Taken together, these findings show that increased expression of adenovirus-mediated SOCS-1 in the lungs of mice significantly protects against hyperoxic lung injury.

Introduction

Acute lung injury (ALI) is a major clinical problem in the United States with an estimated incidence rate of 262,500 patients and 40–50% mortality [1–3]. In experimental animals, detrimental effects of hyperoxia manifest as ALI. Hyperoxia therapy is a necessary part of treatment for patients with acute and chronic cardiovascular and pulmonary diseases [4–8]. However, prolonged exposure to hyperoxia may lead to ALI. ALI is characterized by severe alveolar damage resulting from an acute inflammatory response that leads to immune cell infiltration and edema [9,10]. The fundamental mechanism of this serious condition evolves from an imbalance between proinflammatory and anti-inflammatory cytokines, and the simultaneous induction of apoptosis activators [1,2,11]. Hence, a viable approach to prevent ALI would be to resolve this imbalance. It is known that cytokine signaling is mediated via the Janus kinases–signal transducer and activator of transcription (JAK-STAT) signaling pathway, an important contributor to the production of inflammatory cytokines [12]. The suppressor of cytokine signaling-1 (SOCS-1) protein is a physiological regulator of cytokine production and has been shown to be the most efficient inhibitor of the JAK–STAT pathway [13].

SOCS-1 is an antiapoptotic and potent anti-inflammatory, negative regulator of the IL-6-mediated JAK–STAT signaling pathway [14,15]. It has also been reported that SOCS-1 exerts its protective effects against apoptosis induced by TNF-α, INF-γ, and LPS [16–21]. Our recent report suggests that IL-6 cytoprotection against hyperoxic acute lung injury (HALI) is associated with enhanced SOCS-1 expression [4]. However, the therapeutic role of SOCS-1 under oxidative stress is not yet known.

Apoptosis signal-regulating kinase-1 (ASK-1) is one of the key mitogen-activated protein kinases (MAPKs) required for reactive oxygen species (ROS) and TNF-α induced cell death and inflammation [21]. Recent studies suggest the possibility that inhibitors

Abbreviations: Ad-GFP, GFP adenovirus; Ad-SOCS-1, SOCS-1 adenovirus; AFC, alveolar fluid clearance; ALI, acute lung injury; ASK-1, apoptosis signal-regulating kinase-1; BAL, bronchoalveolar lavage; JAK-STAT, Janus kinases–signal transducer and activator of transcription.

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http://dx.doi.org/10.1016/j.freeradbiomed.2015.03.036
0891-5849/

of ASK-1 have potential benefits in the management of ALI [22–25] and suggest that ASK-1 is significantly activated and involved in HALI [4]. The presented evidence indicates that Ad-SOCS-1 can protect against hyperoxic injury and is associated in the suppression of ASK-1.

Materials and methods

Reagents and antibodies

The following antibodies were used: SOCS-1 (Immuno-Biological Laboratories, Minneapolis, MN), GAPDH (Cell Signaling Technology, Inc., Beverly, MA), ASK-1 (Santa Cruz Biotechnology, Inc., Santa Cruz, CA), and caspase-3 (Cell Signaling Technology, Inc., Beverly, MA). The protein concentration of cell lysates was quantified using a BCA assay kit (Thermo scientific, Rockford, IL). The SOCS-1 adenovirus (Ad-SOCS-1) and GFP adenovirus (Ad-GFP) were gifts kindly provided by Dr. Akihiko Yoshimura (Japan). All other reagents were purchased from Sigma (St. Louis, MO).

Mice

The approval of these animal procedures was obtained from the University of South Florida Institutional Animal Care and Use Committee (IACUC) and all mice were maintained in a specific-pathogen-free animal facility at the University of South Florida. C57BL/6J (wild type, 6 weeks old, 50% male, and 50% female; $n=20$) mice were purchased from Harlan Laboratories (Indianapolis, IN).

Transfection protocols

Cells were treated with phosphate buffered saline (PBS), Ad-GFP, or Ad-SOCS-1 as noted previously [26]. After reaching confluence, cells were transduced with adenoviral stock containing 10^8 plaque forming units (PFU) according to the manufacturer's protocol (Millipore, Billerica, MA). Within 10–13 days, this treatment yielded greater than 95% of stable transductants. Adenoviral vectors were used to attain *in vivo* stable transduction of mice. C57BL/6 mice were intraperitoneally anesthetized with a ketamine/xylazine mixture. Prior to injection, the ventral area of the neck was sprayed with alcohol. A small incision was made in the ventral neck skin area to expose the trachea of each mouse. The adenovirus (10^8 PFU) in 50 μl of PBS was injected into the trachea. The incision was closed with wound closures and the mice were monitored until they recovered from anesthesia. Infected animals were maintained in separate cages for 72 h before hyperoxic exposure. Experimental groups Ad-SOCS-1 ($n=20$), Ad-GFP ($n=20$), and control group PBS ($n=20$) were studied.

Hyperoxia exposure

Six-week-old mice ($n=20$) were placed in cages in a chamber ($75\times50\times50$ cm) and exposed to 100% O_2 for 72 h. The controls were exposed to room air. Concentration of O_2 in the chamber was regulated and monitored with a proOx P100 sensor (BioSpherix) as previously described [2–4].

Bronchoalveolar lavage fluid collection

Mice were anesthetized with an intraperitoneal injection of ketamine/xylazine mixture. After cervical dislocation, the trachea was surgically exposed in the ventral neck area, and a 0.6 mm catheter was inserted into the trachea through a small incision [2,5,27]. Bronchoalveolar lavage (BAL) fluid was collected by perfusing the lungs with sterile PBS as previously described [27]. The BAL fluid perfusion was repeated three times for each mouse. The cell-free BAL fluid was stored at −80 °C until analysis.

Lung perfusion and tissue collection

After BAL fluid collection, the abdominal cavity was opened and lungs were perfused through the right ventricle using 10% formalin in PBS at pH 7.40. The left lobe of the lung was fixed in 0.5 ml of 10% neutral buffered formalin; then it was separated from the cavity for histological processing and paraffin embedding (FFPE) [2,5,27]. The remaining pieces of lungs were stored at −80 °C until analysis. The paraffin-embedded lung tissue sections were stained with hematoxylin and eosin to evaluate the extent of lung injury.

ELISA

Levels of IL-1β (eBioscience, San Diego, CA), IL-6 (BD Bioscience, San Diego, CA), TNF-α (RayBiotech Inc., Norcross, GA), and MCP-1 (eBioscience, San Diego, CA) in BAL fluid were measured using commercial ELISA kits as per the manufacturer's instructions.

Lung injury evaluation

To quantitatively examine lung edema, we recorded wet/dry weight ratios by removing six lungs per group from the hilum as previously described [28]. The lungs were dry blotted and weighed to determine the wet weight. Then the lungs were desiccated overnight by 130 °C incubation in a vacuum oven and reweighted to obtain the dry weight. We then calculated the wet/dry ratio [28]. The remaining portions of the lungs were dissected out carefully, frozen in liquid nitrogen, and stored at −80 °C until analysis.

Alveolar fluid clearance (AFC)

AFC was measured as previously described [29] AFC was calculated by:

$\mathrm{AFC}=[(V_i-V_f)/V_i)]\times 100\%$ $V_f=(V_i\times E_i)/E_f$, where V_i represents the volume of injected albumin solution and V_f represents the volume of the final alveolar fluid and E represents the (E_i) injected and (E_f) final concentrations of the Evans Blue-labeled 5% albumin solution.

Survival study

Mice treated with Ad-SOCS-1 ($n=20$) or Ad-GFP ($n=20$) were exposed to continuous 100% O_2 exposure (hyperoxia) for evaluation of survival. The number of surviving mice was determined at 24-h intervals until the last mouse died.

Analysis of BAL fluid

Entire BAL fluid (~2–3 ml) was centrifuged at 200*g* for 10 min at 4 °C. The supernatants were stored at −80 °C until analysis, and the cell pellets were resuspended with ice-cold sterile PBS (1 ml). The total number of cells in cell suspension was counted using a glass hemocytometer. Aliquots of 100–300 μl of each cell suspension were centrifuged onto glass slides at 800 rpm for 3 min in a cytocentrifuge (Shandon Cytospin 2, Pittsburgh, PA). Cytospinned cells were stained with Diff-Quik stain set (Andwin Scientific, Schaumburg, IL) and differential white blood cell count was performed on a minimum of 200 cells under a microscope at 200× magnification [27].