STAT-3 regulates surfactant phospholipid homeostasis in normal lung and during endotoxin-mediated lung injury

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Ikegami M, Falcone A, Whitsett JA. STAT-3 regulates surfactant phospholipid homeostasis in normal lung and during endotoxin-mediated lung injury. J Appl Physiol 104: 1753–1760, 2008.—Acute lung injury associated with surfactant deficiency remains a major cause of pulmonary morbidity and mortality. Since signal transducer and activator of transcription-3 (STAT-3) plays an important role in protecting respiratory epithelial cells during injury, we hypothesized that STAT-3 may regulate gene expression in type II cells that mediate surfactant phospholipid synthesis. Conditional deletion of Stat-3 in respiratory epithelial cells in the lung of transgenic mice (Stat-3flx/flx mice) decreased surfactant phospholipid synthesis and secretion. Deletion of Stat-3 was associated with decreased expression of Akt2, Srebf-1, and other genes expressed in type II cells that may influence surfactant phospholipid synthesis (Glut-1, Slc34a2, Gpam, Acox2, and Cad2). Stat-3flx/flx mice were more susceptible to intratracheal lipopolysaccharide (LPS). Saturated phosphatidylcholine and surfactant protein B levels were significantly decreased in bronchoalveolar lavage fluid from LPS-treated Stat-3flx/flx mice. Alveolar capillary leak, proinflammatory cytokine expression, and perturbations of lung mechanics caused by LPS were exacerbated after deletion of STAT-3. STAT-3 plays a critical role in the regulation of surfactant lipid synthesis in the normal lung and during lung injury caused by LPS.

Acute lung injury; lipopolysaccharide; cytokine signaling; Akt2; Srebf-1

PULMONARY SURFACTANT is a complex mixture of lipids and proteins that are synthesized and secreted by alveolar type II epithelial cells. Surfactant adsorbs to the air–liquid interface and forms saturated phosphatidylcholine (Sat PC)-rich surfactant films that reduce surface tension in a process that is dependent on surfactant protein (SP)-B (49). While surfactant pool sizes are tightly regulated in the normal lung, the transcriptional mechanisms regulating genes critical for surfactant phospholipid homeostasis are not well understood.

Acute lung injury (ALI) remains a common cause of morbidity and mortality following pulmonary or systemic infection (6). Lipopolysaccharide (LPS) is a constituent of the outer cell wall of gram-negative microorganisms. During bacterial infection, LPS increases capillary permeability, expression of cellular adhesion molecules, proinflammatory cytokines, and chemokines, which associate with ALI (42, 47). Surfactant homeostasis is disrupted in ALI, caused by factors that include a lack of surface-active components, changes in the surfactant composition, and inhibition of surfactant function by serum proteins that leak into the injured alveoli (11, 34). The transcriptional mechanisms that maintain or increase surfactant phospholipids during the acute phase of lung injury and lead to a recovery are poorly understood.

Signal transducer and activator of transcription-3 (STAT-3) is a member of the STAT family of transcription factors that regulates the expression of many acute-phase response genes. STAT-3 is activated by members of the IL-6-like group of proinflammatory cytokines (1, 54). STAT-3 is activated by tyrosine phosphorylation mediated by janus kinases and dimerization, via p-Tyr-SH2 domain interactions that cause nuclear translocation and transcriptional activation of responsive genes (30). Since deletion of the Stat-3 gene before gastrosalvation is lethal (44), the function of STAT-3 in various organs has been examined in cell culture systems and after conditional deletion in transgenic mice models in which STAT-3 is deleted in specific cells (3, 4, 7, 37, 43). STAT-3 is expressed in various cell types in the lung, including alveolar epithelial type II cells. We previously developed transgenic mice in which Cre was conditionally expressed to delete the Stat-3 gene in respiratory epithelial cells of the lung (Stat-3flx/flx mice) (15). While postnatal lung function was maintained, Stat-3flx/flx mice were highly susceptible to hyperoxia (15) and intratracheal (IT) administration of adenosine (27). Increased mortality of Stat-3flx/flx mice was observed following hyperoxia and was associated with decreased SP-B (15). IT adenosine caused severe lung injury in Stat-3flx/flx mice that was associated with increased apoptosis. In the present study, lung injury was induced in Stat-3flx/flx mice by IT injection of LPS. Previous studies by RNA microarray analysis of type II cells isolated from Stat-3flx/flx mice demonstrated significant changes in expression of numerous genes associating with cytoprotection of the lung, including those genes regulating lipid synthesis (27, 52). In the present study, expression of a number of genes related to synthesis of surfactant phospholipids was influenced by deletion of the Stat-3 gene in the normal and injured lung. STAT-3 plays an important role in LPS-induced lung injury, serving to maintain pulmonary homeostasis and repair.

MATERIALS AND METHODS

Transgenic mice with conditional deletion of STAT-3 in respiratory epithelial cells. Triple transgenic mice [SP-C-rtTA tetO]:CMV-CreERT2 (Stat-3flx/flx), herein termed Stat-3flx/flx, were generated as previously reported (15). Stat-3flx/flx mice were kindly provided by Dr. Takeda (Hyogo College of Medicine, Hyogo, Japan) (44). Stat-3flx/flx littermates lacking either rtTA or Cre genes served as controls. These two control line mice were similar, and their lung structure remained normal until they were over 1 yr old. Mice were housed in a pathogen-free, humidity- and temperature-controlled vivarium on a 12:12-h light-dark cycle, in accordance with institutional guidelines.

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There was no serological evidence of pulmonary pathogens or bacterial infections in sentinel mice maintained within the colony. Dams bearing control and Stat-3Δ/Δ mice were maintained on doxycycline in food (625 mg/kg; Harlan Teklad, Madison, WI) from embryonic day 0 until postnatal day 14, resulting in extensive deletion of Stat-3 in respiratory epithelial cells. Mice were then provided normal food. All mice were studied at ages 7–8 wk under protocols approved by the Institutional Animal Care and Use Committee at Children’s Hospital Research Foundation.

Surfactant PC synthesis and secretion. By intraperitoneal injection, Stat-3Δ/Δ and control mice were given 10 μg body wt, containing 1 μCi [%3H]palmitic acid that was stabilized in 0.9% NaCl with 5% human serum albumin (19). Groups of six mice were killed at 8 h after radiolabeled precursor for surfactant phospholipid injection. Bronchoalveolar lavage (BAL) fluid (BALF) was recovered from each animal, and then lung tissue was homogenized in saline. Sat PC was isolated from the BALF and lung homogenate as described below. Radioactivity and amount of phosphorus (14) in isolated Sat PC was measured to determine the incorporation of radiolabeled surfactant precursor into surfactant Sat PC. The percent phospholipid secretion was calculated as the percentage of radioactivity in BALF Sat PC relative to the radioactivity in total lung Sat PC.

Validation of mRNAs. RT-PCR was used to validate changes in several mRNAs related to phospholipid synthesis that were previously detected in Stat-3Δ/Δ mice by RNA microarray analysis (27, 52). Changes in mRNA were determined in type II cells isolated from Stat-3Δ/Δ and controls (n = 5/group). RNA was extracted from isolated type II cells using the RNAeasy Mini Kit (Quiagen, Valencia, CA). cDNA was synthesized by using 5 μg total RNA and high-capacity cDNA Reverse Transcription Kit (Applied Biosystems, Foster City, CA). Quantitative RT-PCR was analyzed using TaqMan gene expression assays (Applied Biosystems). All probes, including the probe for β-actin as the endogenous control, were selected from the list of Applied Biosystems, with the exception of Srebf-1. Twenty-five nanomoles of primer cDNA were used for each sample. Cycle thresholds for β-actin were similar in Stat-3Δ/Δ (19.0 ± 0.4) and control (19.3 ± 0.5) mice (P > 0.05, n = 5/group). The probe for Srebf-1 was designed to bind to the exon-exon junction that is unique to Srebf-1 by using the sequence ATCGGCGCGGAGCTCGGGGGTACGTCGACGCCCTAGGGGATCGCCGACCAGCAGGACCATGAGAATTCTGGAGACATGCAACTCGACGCTACATCAACACAAAGACAGTGAACCTCTGGCTCGTGGCAGCCTCCAGCCAGCCC (40).

Lung injury induced by IT LPS. Mice were anesthetized by 25% isoflurane and intubated orally with a 24-gauge feeding needle, and 10 μg of LPS suspended in 80 μl of 0.9% NaCl were administered IT (18). Mice treated identically with 0.9% NaCl served as experimental control. Mice recovered from anesthesia immediately after IT instillation.

Quantification of STAT-3 mRNA in lung tissue and isolated type II cells. Type II cells were isolated from control and Stat-3Δ/Δ mice using collagenase and differential plating (36). Lung tissue and type II cells were homogenized in TRIzol reagent (Invitrogen, Carlsbad, CA), and the RNA was extracted. After DNase treatment, cDNA was synthesized by using Superscript II. Quantitative RT-PCR for STAT-3 and β-actin mRNAs was performed using Smart Cycler (Cepheid, Sunnyvale, CA), as described before (15).

BALF and lung homogenate samples. At a preassigned time after LPS IT, mice were deeply anesthetized with intraperitoneal pentobarbital sodium (100 mg/kg) and killed by exsanguination. Untreated mice were evaluated as the 0-h group. IT injection of 0.9% NaCl (experimental control) did not influence surfactant contents, lung inflammation, lung morphology, and lung mechanics 0.75 to 16 h after injection, and all were similar to that of 0-h group. Therefore, we have presented 0-h group as the baseline. BALF samples were obtained by pooling five 1-ml aliquots of 0.9% NaCl, which were instilled into the lungs and withdrawn three times to obtain each aliquot. BALF volumes were similar to all groups of mice (control: 4.47 ± 0.03, Stat-3Δ/Δ: 4.43 ± 0.05 ml, n = 20/group). Aliquots of BALF were used to determine Sat PC, inflammatory cell numbers, differential cell counts, SPs, and total protein. After BAL, lungs were homogenized for Sat PC analyses. IL-1β, IL-6, and macrophage inflammatory protein-2 (MIP-2) levels were determined in supernatants of lung
homogenates after centrifugation at 1,500 g for 15 min using quantitative murine sandwich ELISA kits (R&D Systems, Minneapolis, MN).

Sat PC, total phospholipid, surfactant proteins, and total protein. Sat PC was isolated from lipid extracts of BALF and lung homogenates. Lipids were reacted with osmium tetroxide (25), followed by phosphorus measurement (14). Percent Sat PC in BALF relative to total lung Sat PC was calculated. For total phospholipid quantification, phosphorus was measured on the lipid extracts of BALF. The volumes of recovered BALF from all of the groups were similar. Content of SP-A, SP-B, SP-C, and SP-D was determined by Western blot analysis (24) using the same volume BALF for each SP. Immunoreactive bands were detected with enhanced chemiluminescence reagents (Amersham, Chicago, IL), and band intensities were quantified by densitometry (ImageQuant version 5.2, GE Healthcare, Piscataway, NJ). Total protein in aliquots of BALF was measured by the method of Lowry et al. (23) and calculated as milligrams per kilogram body weight.

Lung histology. Lungs (3 mice/group) were inflation fixed with 4% paraformaldehyde in PBS at 25 cm H2O and immersed in the same fixative. Tissue was fixed overnight, washed with PBS, and dehydrated in a series of alcohols, and six lung lobes were separated and embedded in paraffin. Tissues were stained with hematoxylin and eosin for histology. Six areas of lung tissue in a ~40 field of view (0.024 mm²) were randomly selected for each six lung lobes from each animal. Lung inflammation was evaluated as 0 (no inflammation) to 3 (severe inflammation with increased inflammatory cells in the airways) (29) in increments of 0.5.

Lung mechanics. Four hours after LPS or saline IT, lung mechanics were studied in tracheostomized mice under anesthesia by intraperitoneal injection of ketamine (20 mg/ml) and xylazine (2 mg/ml) in proportion to body weight (0.1 ml/10 g). Mice were ventilated with a tidal volume of 8 ml/kg at a rate of 450 breaths/min (8) and a positive end-expiratory pressure of 2 cm H2O by a computerized FlexiVent System (SCIREQ Scientific Respiratory Equipment, Montreal, Quebec, Canada). This apparatus allows for accurate measurement of volume by using the position of the ventilator piston and pressure in the cylinder. After mechanical ventilation for 2 min, two isolated measurements were performed. For the initial measurement, a sinusoidal 1-Hz oscillation was applied to the tracheal tube. The single-compartment model was fit to these data using a multiple linear regression to calculate dynamic resistance, elastance, and compliance of the airways. For the second measurement, a 16-s forced oscillatory signal containing frequencies between 0.25 and 19.625 Hz was imposed.
applied to the tracheal tube. Mechanical input impedance of the respiratory system was calculated, and a model containing a constant-phase tissue compartment was fit to input impedance to evaluate tissue damping, tissue elastance, and tissue hysteresivity (12, 15). The respiratory system was calculated, and a model containing a constant-phase tissue compartment was fit to input impedance to evaluate tissue damping, tissue elastance, and tissue hysteresivity (12, 15). The respiratory system was calculated, and a model containing a constant-phase tissue compartment was fit to input impedance to evaluate tissue damping, tissue elastance, and tissue hysteresivity (12, 15).

Statistical analysis. Results were presented as means ± SE. Two group comparisons were carried out using unpaired Student’s t-test. Comparisons among groups were assessed by ANOVA with Tukey’s tests used for post hoc analyses. Scoring of lung histology data is presented as the median score followed by a Mann-Whitney rank-sum test. Differences were considered significant at the 5% level.

RESULTS

Decreased surfactant Sat PC synthesis and secretion in Stat-3ΔΔ mice. As shown in our laboratory’s previous study (15), adult Stat-3ΔΔ mice had a reduced level of Sat PC in BALF and total lung (BALF + lung tissue) (P < 0.05) at baseline. Sat PC in lung tissue after BALF was similar to that of control mice (Fig. 1A), indicating that the reduction in Sat PC was primarily related to decreased alveolar content. In Stat-3ΔΔ mice, percentage of Sat PC in BALF relative to the total lung was significantly reduced to 53% of that in control mice (Fig. 1B).

Body weights were similar in control and Stat-3ΔΔ mice (control: 23.2 ± 0.8, Stat-3ΔΔ: 22.5 ± 0.7 g, n = 24/group). Mice were given body weight-adjusted doses of [3H]palmitic acid by intraperitoneal injection, and the amount of labeled Sat PC was measured in BALF and lung tissue after BAL 8 h after injection. Previous studies demonstrated that uptake of [3H]-labeled Sat PC by macrophages and type II cells was minimum 8 h after [3H]palmitic acid injection. This time point was considered optimal to measure the net incorporation of precursors into Sat PC and secretion of labeled Sat PC into the alveoli (19). Incorporation of radiolabeled precursor ([3H]palmitic acid) into Sat PC in BALF, lung tissue, and total lung (Fig. 1C) and percent secretion calculated from [3H]Sat PC in BALF relative to the radioactivity in total lung Sat PC (Fig. 1D) were significantly decreased in Stat-3ΔΔ mice. PC secretion during 3 h was determined in vitro using cultured type II cells isolated from control and Stat-3ΔΔ mice (n = 11 plate/group). Percent secretion of [3H]choline-labeled PC from type II cells was 30% lower in Stat-3ΔΔ mice (6.1 ± 0.4%) than the controls (8.5 ± 0.8%, P < 0.01). Both synthesis and secretion of Sat PC were decreased by deletion of Stat-3 in respiratory epithelial cells.

Deletion of Stat-3 decreased expression of genes regulating surfactant phospholipid synthesis. RNA microarray analysis of type II cells isolated from Stat-3ΔΔ mice demonstrated decreased expression of a number of genes related to surfactant phospholipid synthesis (27). Akt2, Srebf-1, Acox2, Glut1, Cds2, Slc34a2, and Gpmn in RNAs were significantly decreased in type II cells from Stat-3ΔΔ mice (Fig. 2), demonstrating the important role of STAT-3 in regulation of key genes mediating surfactant phospholipid synthesis.

Changes in STAT-3 mRNA after LPS. In control mice, expression of STAT-3 mRNA was increased in both lung tissue (Fig 3A) and type II cells (Fig. 3B) after IT LPS injection. In control mice, expression of STAT-3 mRNA was increased in both lung tissue (Fig 3A) and type II cells (Fig. 3B) after IT LPS injection.

**Fig. 5.** Stat-3 influenced surfactant protein (SP)-B after LPS exposure. B: SP-B was decreased in BALF from Stat-3ΔΔ mice after LPS injection. In contrast, SP-A (A), SP-C (C), and SP-D (D) were increased in control and Stat-3ΔΔ mice after LPS exposure. Values are means ± SE; n = 4 mice/group. *P < 0.05 vs. control mice.

**Fig. 6.** Protein leak and inflammation after IT LPS. A: protein in BALF was increased 2 h after LPS injection in control and Stat-3ΔΔ mice, consistent with increased protein permeability. Protein in BALF was higher in Stat-3ΔΔ mice than in control mice 16 h after LPS. B: inflammatory cells in BALF were increased 16 h after LPS IT in control mice; inflammatory cells were increased in Stat-3ΔΔ mice as early as 2 h after LPS IT. C: neutrophils were increased in BALF from Stat-3ΔΔ mice 0.75 and 4 h after LPS injection compared with controls. D: macrophages in BALF were increased in Stat-3ΔΔ, but not control, mice 16 h after LPS. Values are means ± SE; n = 4 mice/group. *P < 0.05 vs. 0 h. 'P < 0.05 vs. control mice.
injection and returned to the baseline 16 h later. As shown in our previous study, STAT-3 was permanently deleted in Stat-3/H9004 mice from intrapulmonary respiratory epithelial cells after administration of doxycycline to the dam (15). In lungs of Stat-3/H9004 mice, STAT-3 mRNA was 50% of that in controls and was increased after LPS injection (Fig. 3A). In contrast, STAT-3 mRNA in isolated type II cells from Stat-3/H9004 mice was only 10% of that in control mice (Fig. 3B). The purity of isolated type II cells from mice was typically >90%, as assessed by modified Papanicolaou stain, and immunostaining for pro-SP-C (36). The majority of contaminating cells were alveolar macrophages in which STAT-3 was detected. In this system, relatively complete STAT-3 gene deletion occurred in type II cells, whereas expression of Stat-3 in other cells was not altered.

Surfactant homeostasis in Stat-3/H9004 mice after IT LPS. Sat PC and phospholipid in BALF was increased in control mice after IT LPS, consistent with acute response to lung injury (Fig. 4, A and C). In contrast, Sat PC in Stat-3/H9004 mice BALF did not increase until 16 h after LPS injection and was significantly lower than in control mice. The decrease in Sat PC was primarily in the alveolar pool. Sat PC in lung homogenates from control and Stat-3/H9004 mice was similar (Fig. 4B).

As shown previously (15), the basal levels of SP-A, SP-B, SP-C, and SP-D in BALF were unaffected by the deletion of Stat-3 under no stress, 0-h group (Fig. 5). Expression of SP-A, SP-B, and SP-C mRNAs was similar in control and Stat-3/H9004 mice (15). Two hours after LPS exposure, SP-A and SP-D in BALF were increased in both control and Stat-3/H9004 mice. In control mice, SP-B content was increased more than twofold following LPS exposure. In Stat-3/H9004 mice, SP-B was markedly decreased at 2 h, a finding similar to our previous studies with Stat-3/H9004 mice after hyperoxic lung injury (15). In contrast, SP-C was increased following LPS exposure in both Stat-3/H9004 and control mice, demonstrating the distinct regulation of Sat PC and SP-B, compared with SP-C.

Stat-3/H9004 mice are susceptible to IT LPS. Total protein (Fig. 6A), inflammatory cells (Fig. 6B), and neutrophils (Fig. 6C) in BALF were increased 2 h after LPS injection in both control and Stat-3/H9004 mice (P < 0.05 vs. 0 h). Total protein in BALF from Stat-3/H9004 mice was higher than that of control mice.

Increased proinflammatory cytokines after LPS exposure. LPS induced IL-1β (A), IL-6 (B), and macrophage inflammatory protein-2 (MIP-2; C) in both control and Stat-3/H9004 mice at all of the hours studied (P < 0.05 vs. 0 h). Values are means ± SE; n = 4 mice/group. Cytokine levels were higher in Stat-3/H9004 mice than in control mice, 16 h after LPS. *P < 0.05 vs. control mice.

Fig. 7. Increased proinflammatory cytokines after LPS exposure. LPS induced IL-1β (A), IL-6 (B), and macrophage inflammatory protein-2 (MIP-2; C) in both control and Stat-3/H9004 mice at all of the hours studied (P < 0.05 vs. 0 h). Values are means ± SE; n = 4 mice/group. Cytokine levels were higher in Stat-3/H9004 mice than in control mice, 16 h after LPS. *P < 0.05 vs. control mice.

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Fig. 8. Lung morphology. A representative photographs of lung from Stat-3/H9004 mice (A–D) and control mice (E and F) are provided. For Stat-3/H9004 mice: score 0, 0 h (A); score 1, 16 h after LPS (B); score 2, 4 h after LPS (C); score 3, 4 h after LPS (D). For control mice: score 0, 4 h after LPS (E); score 1, 4 h after LPS (F). G: the median injury scores were increased in Stat-3/H9004 mice treated with LPS. *P < 0.05 vs. control mice by Mann-Whitney rank-sum test. n = 3 mice/group. There were 6 areas/lobe and 6 lobes/mouse analyzed.
(P < 0.05) 16 h after LPS injection, (Fig. 6A), consistent with the sustained increase in protein permeability in the Stat-3Δ/Δ mice after LPS. Numbers of macrophages in BALF of control mice were not influenced by LPS injection (P > 0.05), whereas macrophages were significantly increased in Stat-3Δ/Δ mice 16 h after LPS injection (Fig. 6D). Proinflammatory cytokines in lung homogenates, including IL-1β (Fig. 7A), IL-6 (Fig. 7B), and MIP-2 (Fig. 7C), were low at 0 h and were increased after LPS injection in both control and Stat-3Δ/Δ mice (P < 0.05 vs. 0 h). IL-1β, IL-6, and MIP-2 concentrations peaked at 2 or 4 h and decreased 16 h after injection in control mice. In contrast, IL-1β, IL-6, and MIP-2 in Stat-3Δ/Δ mice were higher than in control mice 16 h after LPS injection, suggesting delayed recovery from lung inflammation.

Severity of lung injury after LPS injection in Stat-3Δ/Δ mice was demonstrated histologically. Morphological lung injury was evaluated blindly as 0 (no inflammation) to 3 (severe inflammation). Representative histology for each score is shown in Fig. 8 (A–D for Stat-3Δ/Δ, E and F for control mice). Lung inflammation was more severe in Stat-3 gene-deleted lung than in control mice lung (Fig. 8G).

Pulmonary dysfunction in Stat-3Δ/Δ mice after LPS injection. IT LPS injection did not influence lung mechanics in control mice. While pulmonary mechanics were unaltered in Stat-3Δ/Δ mice under the nonstressed condition, IT injection of LPS resulted in a marked decline in pulmonary function (Fig. 9). Lung compliance was decreased in association with a significant increase in airway resistance, tissue damping, and elastance, consistent with deterioration of surfactant components and tissue edema in Stat-3Δ/Δ mice. No differences in hysteresivity were detected, indicating that coupled viscoelasticity of the lung was not influenced in LPS-treated Stat-3Δ/Δ mice.

**DISCUSSION**

The balance among surfactant synthesis, secretion, catabolism, and recycling precisely regulates surfactant pool sizes. Herein, we show that deletion of Stat-3 in respiratory epithelial cells decreased surfactant synthesis and surfactant secretion. In the present study, both Srebf-1 and Akt2 mRNAs were significantly decreased by deletion of Stat-3 in type II epithelial cells in vivo. Sterol regulatory element binding factor, Srebf-1, a gene encoding Srebf-1c, regulates transcription of genes influencing fatty acid and steroid biosynthesis in a process that is known to be influenced by Stat-3 via Akt2 (32, 35, 51). A diagram outlining pathways mediating de novo synthesis of surfactant phospholipid (5) is shown in Fig. 10. A number of
the components of this pathway were altered after deletion of Stat-3 in respiratory epithelial cells. Alveolar type II cells utilize phosphate and glucose as a substrate for phospholipid synthesis. Glucose transport is predominantly governed by Glut-1, a member of the glucose transport family (28). Glucose transport regulated by Glut-1 is a rate-limiting step in glucose utilization by isolated rat type II cells (33). Glut-1 is the primary glucose transporter in the lung (46). Scl34a2, the gene for type II bNa/Pi cotransporter, is known to control phosphate reabsorption in the renal proximal tubule. Scl34a2 is highly expressed in epithelial type II cells, where it influences phosphate transport associated with surfactant phospholipid synthesis (13, 45). The initial step de novo of surfactant is the catalysis required to glycerolipid synthesis mediated by glycerol-3-phosphate acyltransferase (Gpam). Acox2 encodes acyl-coenzyme A oxidase 2, which catalyzes the formation of phosphatic acid. Cds2 encodes CDP-diacylglycerol synthase, an enzyme that catalyzes the formation of CDP-diacylglycerol from phosphatidic acid (5). Expression of these key genes regulating phospholipid synthesis was significantly decreased in type II cells isolated from Stat-3Δ/Δ mice. Immunohistochemistry for pro-SP-C, a protein expressed only in type II epithelial cells, revealed that there were no changes in the number of type II cells caused by deletion of Stat-3 (15).

Biochemical pathways regulating de novo synthesis of surfactant phospholipid are well known, although the precise transcriptional mechanisms regulating their expression in the lung have not been identified. The present study demonstrates that Stat-3 plays a critical role in surfactant homeostasis in normal and injured lung, regulating expression of genes indicating phospholipid synthesis. Decreased surfactant secretion in Stat-3Δ/Δ mice is likely to be caused, at least in part, by decreased ATP-binding cassette A3 (Abca3) expression. Abnormal ultrastructure of lamellar bodies, the surfactant storage organelle, was observed in Stat-3Δ/Δ mice previously (26). Abca3 is present in the limiting membranes of the lamellar bodies; mutations in the Abca3 gene cause respiratory distress in newborn infants associated with altered surfactant metabolism and abnormal ultrastructure of lamellar bodies (38, 41).

Previous studies demonstrated that lung compliance and oxygenation were not influenced until >50% of surfactant was removed from alveolus by BAL (16, 22), indicating a reserve of alveolar surfactant. Consistent with these observations, a 40% decrease in BALF Sat PC in Stat-3Δ/Δ mice did not influence lung function at baseline. Thus STAT-3 independent mechanism(s) suffice to maintain adequate Sat PC to survive under nonstress conditions. Pulmonary infection caused by gram-negative bacteria is commonly associated with ALI (42, 47). Microbial toxins and LPS, rather than the actual bacterium, can initiate cellular and humoral inflammatory responses. The present study demonstrates that, while respiratory epithelial cell-specific deletion of Stat-3 did not alter lung morphogenesis or function, STAT-3 was required for surfactant homeostasis, including Sat PC synthesis and secretion. SP-B in BALF was significantly decreased in Stat-3Δ/Δ mice by LPS injection. Using the conditionally expressing SP-B in Sftpb−/− mice, we have previously shown that the loss of SP-B was sufficient to perturb surfactant function, initiate proinflammatory cytokine expression (IL-6, IL-1β, and MIP-2), and disrupt pulmonary mechanics in the adult lung (20). STAT-3 activates Sftpb expression in vivo and in vitro (53). The normal increase in Sat PC in acute phase of lung injury (2, 9, 31, 48, 50) was not seen in Stat-3Δ/Δ mice, and Sat PC in BALF was significantly lower in Stat-3Δ/Δ mice. The lower concentration of Sat PC in BALF renders surfactant susceptible to inhibition by plasma proteins (17) that may play a role in the increased LPS-induced lung injury seen in Stat-3Δ/Δ mice. In the absence of STAT-3, lung injury induced by LPS was more severe than in control mice, with decreased Sat PC and SP-B leading to respiratory compromise.

Although an increase in activated STAT-3 in ALI has been known (10, 39), the specific roles of Stat-3 in respiratory epithelial cells remain unclear. ALI is a frequent life-threatening disease, resulting in 25–50% mortality in adults and children. ALI is associated with surfactant deficiency and dysfunction (11, 34) caused by factors, including lack of surface-active material; changes in phospholipid, fatty acid, and neutral lipids; and inhibition of surfactant activity related to inflammation and leakage of cellular and serum proteins into the alveolar compartment. In the normal adult lung, surfactant phospholipids and SP-B are increased during the acute phase of lung inflammation in mice, rats, and rabbits (2, 9, 31, 48, 50) and subsequently decrease (21). This induction of surfactant homeostasis during the acute phase of lung injury is required for maintenance of lung function during, and recovery from, ALI. From a clinical perspective, activation of pathways maintaining or increasing STAT-3 in respiratory epithelial cells may provide a strategy for protection of the lung during injury and prevent the lung from ALI.

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