ErbB4 deletion leads to changes in lung function and structure similar to bronchopulmonary dysplasia

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ErbB4 deletion leads to changes in lung function and structure similar to bronchopulmonary dysplasia. Am J Physiol Lung Cell Mol Physiol 294: L516–L522, 2008. First published January 18, 2008; doi:10.1152/ajplung.00423.2007.—Neuregulin is an important growth factor in fetal surfactant synthesis, and downregulation of its receptor, ErbB4, impairs fetal surfactant synthesis. We hypothesized that pulmonary ErbB4 deletion will affect the developing lung leading to an abnormal postnatal lung function. ErbB4-deleted lungs of 11- to 14-wk-old adult HER4heart mice, rescued from their lethal cardiac defects, were studied for the effect on lung function, alveolarization, and the surfactant system. ErbB4 deletion impairs lung function and structure in HER4heart mice resulting in a hyperreactive airway system and alveolar simplification, as seen in preterm infants with bronchopulmonary dysplasia. It also leads to a downregulation of surfactant protein D expression and an underlying chronic inflammation in these lungs. Our findings suggest that this animal model could be used to further study the pathogenesis of bronchopulmonary dysplasia and might help design protective interventions.

surfactant; lung development; alveolar simplification; hyperreactive airway system

SURFACTANT SYNTHESIS AND ALVEOLARIZATION are critical parts of fetal lung development. Interactions between the mesenchyme and type II epithelial cells play a crucial role in the onset of fetal surfactant synthesis (30). Neuregulin takes part in this process (8). Its receptor, ErbB4, is the preferred heterodimerization partner in type II epithelial cells (40), and downregulation of ErbB4 reduces surfactant synthesis in fetal type II epithelial cells (41). ErbB4 is the most recently described (23) member of the epidermal growth factor receptor (EGFR)-like subfamily of receptor tyrosine kinases that consists of ErbB1, ErbB2, ErbB3, and ErbB4. ErbB4 knockout animals die at embryonic day 11 due to a defective development of the myocardial trabeculae system (10). We hypothesized that deletion of ErbB4 will affect fetal and postnatal lung development, specifically in ways that might affect the surfactant system and the alveolarization. The knockout animal model for ErbB4 cannot be used to explore the role of ErbB4 in late fetal lung development. We therefore used a transgenic cardiac rescue model where the lethal heart defect was rescued by expressing ErbB4 under a cardiac-specific myosin promoter. These HER4heart mutants reach adulthood, are fertile, and show defects in the neurological and mammary systems (34). Here, we present that ErbB4 deletion leads to anatomic and functional changes in the lung.

MATERIALS AND METHODS

Rabbit anti-human surfactant protein (SP)-A, rabbit anti-sheep SP-B, rabbit anti-human pro-SP-C, and rabbit anti-mouse SP-D antibody were obtained from Chemicon (Hofheim, Germany); EcoRI restriction enzyme was obtained from New England Biolabs (Frankfurt am Main, Germany); digoxigenin (DIG) labeling kit (SP6/T7), T7 RNA polymerase, anti-DIG alkaline phosphatase, proteinase K, blocking reagent, and BM Purple were from Roche Diagnostics (Mannheim, Germany); SuperFrost Plus microscopic slides were from Menzel-Glaser (Braunschweig, Germany); RNAsay MiniElute Cleanup Kit was from Qiagen (Hilden, Germany); maleic acid, diethyl pyrocarbonate, polyvinylpyrrolidone, and ethylenediaminetetraacetic acid disodium dihydrate were obtained from Sigma-Aldrich (Steinheim, Germany); mouse monoclonal anti-actin clone AC-40 was obtained from Sigma (St. Louis, MO); goat anti-rabbit IgG (horse-radish peroxidase-labeled) and goat anti-mouse IgG (horse-radish peroxidase-labeled) were from Zymed Laboratories (Invitrogen, Carlsbad, CA); Precision Plus Protein Dual Color Standards were from Bio-Rad (Hercules, CA); Protran nitrocellulose transfer membrane was from Schleicher & Schuell BioScience (Keene, NH); and Western Lightning Chemiluminescence Reagent Plus (ECL) was from PerkinElmer Life Sciences (Boston, MA). Osmium tetroxide and crystalline were obtained from Paezel and Lorei (Frankfurt, Germany), uranyl acetate dihydrate was from Merck (Darmstadt, Germany), and glycyl ether 100 (epon) was obtained from Serva (Heidelberg, Germany).

Transgenic ErbB4-deleted mice, rescued from their lethal cardiac defects by expressing human ErbB4 (HER4) cDNA under the cardiac-specific α-myosin heavy chain promoter (34), were kindly provided by Dr. Carmen Birchmeier in agreement with Dr. Martin Gassmann. Animal experiments were performed on adult HER4heart mice (11–14 wk old), and heterozygote siblings were used as controls since heterozygote breeding revealed a low number of wild-type animals. All animals were housed in a pathogen-free animal facility at Hannover Medical School, and the protocols were approved by the appropriate governmental and institutional authorities at Hannover Medical School.

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Invasive measurement of pulmonary function. Mice were anesthetized with 1.5% halothane-30% oxygen by inhalation and orotracheally intubated. The technique for invasive and repetitive lung function measurement used in this study has been described recently (11, 14). Briefly, two intubated, spontaneously breathing animals were placed in supine position in temperature-controlled body plethysmographs (type 871; HSE-Harvard Apparatus, March-Hugstetten, Germany). The orotracheal tube was directly attached to a pneumotachograph (HSE-Harvard Apparatus) connected to a differential pressure transducer (Validyne DP 45-14, HSE-Harvard Apparatus) to determine tidal flow. Transpulmonary pressure (Ptp) and tidal flow signals over an entire breath cycle using HEM (Notocord, Croissy, France). Airway responsiveness was assessed as an increase in RL in response to aerosolized 3.5 software (Notocord, Croissy, France). Airway responsiveness was assessed as an increase in RL in response to aerosolized 3.5 software (Notocord, Croissy, France). Airway responsiveness was assessed as an increase in RL in response to aerosolized 3.5 software (Notocord, Croissy, France). Airway responsiveness was assessed as an increase in RL in response to aerosolized 3.5 software (Notocord, Croissy, France). Airway responsiveness was assessed as an increase in RL in response to aerosolized 3.5 software (Notocord, Croissy, France).

Tissue processing. After intratracheal instillation with 4% paraformaldehyde (PFA) and 0.1% glutaraldehyde in 0.2 M HEPES buffer (pH 7.35) using an instillation device (pressure of 20 cmH2O) and 4 h postfixation in the same fixation solution, two blocks from the right lung were embedded in paraffin and used for in situ hybridization. The left lung was cryoprotected in glucose solution and cryofixed for morphometry and immunohistochemical analysis.

Stereological analysis. Stereological analysis was carried out by one person, blinded to the genotype of the animal, on a Nikon Eclipse 80i microscope (Tokyo, Japan) using the Stereo Investigator version 6 software (MicroBrightField).

Three to five 10-μm-thick hematoxylin-eosin-stained sections from each animal were analyzed using a grid of test points and cycloid lines. The total number of points and intersections in each lung were >1,000. Morphometric parameters, such as surface density of alveoli, volume density of alveoli and alveolar septae, volume density of alveolar ducts and their septa, arithmetic barrier thickness of alveolar septae and septae of alveolar ducts, and size of alveoli and alveolar ducts, were calculated according to the point and intersection counting (1, 37).

The number of alveoli were estimated via a method slightly modified from Ochs et al. (22). Therefore, the optical fractionator of the Stereo Investigator system was used. Only alveoli with closed walls that come into the focus of the test field within the 3-μm height of the optical dissector were counted including the counting frame on a total of six to seven 40-μm-thick sections from the left lung of each animal. Maximal diameter of counted alveoli was ~100 μm. At least 200 alveoli were counted in each lung.

Structural analysis by conventional electron microscopy. Processing of tissue for conventional electron microscopy was done as previously described (9). Briefly, after primary fixation in 4% PFA, lung tissue blocks were washed, stained overnight with half-saturated aqueous uranyl acetate, dehydrated in ascending series of acetone, and embedded in Epon. After polymerization at 60°C, ultrathin 70-μm sections were cut (Reichert-Jung Ultracut E; Vienna, Austria) and stained with lead citrate and uranyl acetate. Sections were evaluated using an EM10 electron microscope (Zeiss; Oberkochen, Germany).

Percentages of test fields containing granulocytes were estimated by systematic random counting of neutrophils and eosinophils.

Surfactant protein expression analysis. Lungs were frozen in liquid nitrogen, and Western blot analyses were performed as described previously (8). Protein expression was analyzed from densitometric readings. Immunohistochemistry was performed on 10-μm-thick sections as described previously (28) after endogenous peroxidase was quenched with 3% peroxide for 10 min. SP-B in situ hybridization on 10-μm-thick paraffin sections was done according to a protocol described previously (18) using riboprobes synthesized from mouse SP-B cDNA kindly provided by Dr. J. Whitsett.

Real-time PCR for SP-A, SP-B, SP-C, and SP-D were done as previously described (12). Primers used for real-time PCR are listed in Table 1. Actin was used as internal control to normalize the surfactant protein cDNA levels. The difference in the threshold cycle (DCT) of the surfactant protein and actin genes was determined. DCT values are inversely proportional to the levels of surfactant protein mRNA.

Statistics. Data are presented as means ± SE if not indicated otherwise. Statistical significance was noted for P values <0.05 using the Student’s t-test for the data, which follow the normal distribution, and the Mann-Whitney U-test otherwise.

RESULTS

Pulmonary function. Baseline lung function parameters, such as tidal midexpiratory flow and respiratory frequency, were significantly reduced in HER4heart−/− animals to 1.18 ± 0.12 ml/s (P = 0.02) and 148 ± 4.3 breaths/min (P = 0.01), respectively, with 1.59 ± 0.12 ml/s and 168 ± 6.6 breaths/min in heterozygote HER4heart+/− control animals (Table 2). MCh provocation led to a significant higher resistance (RL) in knockout mice compared with control animals at MCh doses of 0.125, 0.5, and 1.0 μg, increasing from 10.3% ± 4.7%, 25.8% ± 8.1%, and 44.5% ± 11.8% in control animals to 31.9% ± 8.2% (P = 0.034), 49.1% ± 5.8% (P = 0.018), and 69.6% ± 7.9% (P = 0.047), respectively, above baseline values in HER4heart−/− mice. At MCh doses of 0.0625, 0.25, 2.0, and 4.0 μg, the more pronounced increase of RL in HER4heart−/− mice compared with control animals fell short of statistical significance (Fig. 1). ANOVA testing revealed sig-
significant differences (P < 0.0001) of the RL values of the MCh stages vs. the baseline RL for both the HER4heart+/− and the HER4heart+/− control group. In summary, MCh exposure caused a dose-related increase of RL in HER4heart+/− mice being on a higher level compared with control mice. This shows that pulmonary ErbB4 deletion leads to a significant airway hyperresponsiveness.

**Stereological analysis.** Volume density and thickness of alveolar septa were significantly increased to 120.13% ± 4.99% (P = 0.02) and 120.22% ± 4.82% (P = 0.01), respectively, compared with HER4heart+/− control animals (Fig. 2). In HER4heart+/− mice, the volume density of the ductal space was significantly decreased to 91.06% ± 7.54% (P = 0.03) compared with HER4heart+/− control animals (Table 3).

**Alveolar count.** The number of alveoli in HER4heart+/− lungs (n = 9) was significantly decreased by 20% ± 5% (P = 0.018) compared with HER4heart+/− control lungs (n = 6). Taken together with the stereological results, pulmonary ErbB4 deletion also leads to an alveolar simplification.

**Electron microscopy.** Differences in lamellar body structure were not detected by electron microscopy in HER4heart+/− lungs. However, the number of test fields containing granulocytes was significantly increased (P = 0.016) in HER4heart+/− lungs (n = 4) to 7.65% ± 0.56% compared with 4.52% ± 0.66% in control lungs (n = 5) (Fig. 3), suggesting a chronic inflammatory process in these ErbB4-deleted lungs.

**Surfactant protein expression.** SP-A, SP-B, and SP-C protein expression were similar for both genotypes studied, revealing 105% ± 9.4% (P = 0.5) for SP-A, 101% ± 2.3% (P = 0.74) for SP-B, and 99% ± 2.1% (P = 0.47) for SP-C in HER4heart+/− lungs (n = 11) compared with lungs of control animals (n = 13). However, there was a significant decrease in expression of SP-D to 91% ± 3% (P = 0.017) in HER4heart+/− lungs (Fig. 4B). Immunohistochemical SP-B expression studies confirmed the Western blot results.

**Surfactant protein mRNA expression.** SP-B mRNA expression studied by in situ hybridization confirmed similar expression of this protein independent of the genotype (data not shown). Real-time PCR measurements for SP-A (the difference of the samples DCT and the baseline, DDCt = 0.3 ± 0.4; P = 0.5), SP-B (DDCt = −0.23 ± 0.3; P = 0.6), and SP-C (DCT = −0.7 ± 0.3; P = 0.2) did not show significant differences between HER4heart+/− and HER4heart+/− control lungs, whereas SP-D mRNA expression was significantly decreased in HER4heart+/− (DDCt 0.6 ± 0.3; P = 0.045) compared with HER4heart+/− control lungs (Fig. 5), confirming our protein evaluations.

**DISCUSSION**

Neuregulin and its receptor, ErbB4, play prominent roles in fetal type II cell maturation (8, 40), underlining the strong association of ErbB4 and fetal tissue differentiation (31). Therefore, we hypothesized that ErbB4 is involved in the regulation of normal lung development. We used a transgenic animal model where the embryonic lethality of ErbB4 knockout mice is rescued by expressing the ErbB4 receptor under a cardiac-specific promoter (34). Here, we show for the first time that pulmonary ErbB4 deletion leads to a hyperreactive airway system and to alveolar simplification, a phenotype similar to bronchopulmonary dysplasia (BPD) in preterm infants. Interestingly, SP-D expression is downregulated, and there are signs of an underlying chronic inflammation in these lungs.

At birth, mouse lungs are at the saccular stage of the pulmonary development, and alveoli formation occurs mainly after birth (3). The pulmonary sacs are divided through serial septation processes into alveoli, increasing the gas exchange surface of the lung. Our morphological observations in the lung tissue, specifically the decrease of alveolar number, increase in thickness and volume density of alveolar septa, and the decrease of the alveolar ductal space, indicate lung immaturity with inhibited formation of secondary alveolar septae and can
be summarized as alveolar simplification. However, a combined effect of decreased formation of secondary septa and progressive destruction of parenchyma might be present in these animals. These morphological changes are similar to the changes observed in the lungs of the newborn mice after prolonged exposure to 85% oxygen. Hyperoxia leads to a similar picture seen in BPD lungs, showing decreased alveolar septation, an increase in terminal air space size, and lung fibrosis. It also leads to an increased number of inflammatory cells in lung tissue and in bronchoalveolar lavage fluid (36). Although hyperoxia and barotrauma induce BPD (4), signs of impaired alveolarization can be seen in the lungs of very immature baboons even in the absence of marked hyperoxia and high ventilation settings (5). In the ErbB4-deleted lungs, the size of alveoli was not increased, but all other morphometric parameters resemble those of premature infants suffering from an interruption of normal lung development after the exposure to cytokines or glucocorticoids (24, 39) ultimately leading to the clinical picture of BPD (16). Similarly, exposure of fetal sheep to intra-amniotic endotoxin or IL-1α leads to a decreased alveolarization and microvascular injury (15). VEGF blockade in newborn rats decreases lung angiogenesis and impairs alveolar development, mimicking BPD (33).

Young adults who have been born preterm have a higher prevalence of asthma and respiratory symptoms (35). Airway hyperresponsiveness in preterm born infants is mostly seen without any inheritance of allergy (13), and it remains unclear whether the anatomic changes are the pathological correlate for these symptoms. In agreement with these observations in humans, we found an association of airway hyperresponsive-

Table 3. Morphometric parameters in the lungs of control HER4heart+/− compared with HER4heart−/− mice

<table>
<thead>
<tr>
<th>Morphometric Parameters</th>
<th>% of Family-Specific Controls</th>
<th>P</th>
</tr>
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<tbody>
<tr>
<td>Volume density of parenchyma</td>
<td>100.5±0.58</td>
<td>0.12</td>
</tr>
<tr>
<td>Volume density of nonparenchyma</td>
<td>93.51±9.3</td>
<td>0.44</td>
</tr>
<tr>
<td>Thickness of alveolar septa</td>
<td>120.22±4.82*</td>
<td>0.01†</td>
</tr>
<tr>
<td>Volume density of alveolar septa</td>
<td>120.13±4.99*</td>
<td>0.02†</td>
</tr>
<tr>
<td>Volume density of alveolar space</td>
<td>106.83±4.70</td>
<td>0.45</td>
</tr>
<tr>
<td>Surface density of alveoli</td>
<td>96.16±2.41</td>
<td>0.12</td>
</tr>
<tr>
<td>Size of alveoli</td>
<td>107.44±3.60</td>
<td>0.08</td>
</tr>
<tr>
<td>Volume density of alveolar ductal space</td>
<td>91.06±7.54*</td>
<td>0.03†</td>
</tr>
<tr>
<td>Size of alveolar ducts</td>
<td>96.25±3.53</td>
<td>0.68†</td>
</tr>
<tr>
<td>Volume density of alveolar ductal septa</td>
<td>96.24±6.17</td>
<td>0.34</td>
</tr>
<tr>
<td>Thickness of alveolar ductal septa</td>
<td>106.34±4.93</td>
<td>0.47</td>
</tr>
</tbody>
</table>

Values are means ± SE. *P < 0.05; †P value of Student’s t-test (otherwise, Mann-Whitney U-test).
leads us to the speculation that the minimal SP-D deficiency in normally developed alveoli in these animals. This observation enlarges the air spaces (2, 17), implying remodeling of lungs at birth, and postnatal macrophage influx leads to an foamy macrophages (17). Also, SP-D null mice have normal combined with giant lamellar bodies and the occurrence of tional described hypertrophy and hyperplasia of type II cells was measured and normalized to the HER4 heart. These results are in agreement with our findings in the HER4null mice showing signs of postnatal inflammation (2, 17), a chronic ongoing inflammatory process in those lungs. SP-D expression in ErbB4-deleted lungs confirm the presence of a chronic ongoing inflammatory process in the ErbB4 null mice cannot account for the anatomic alterations seen in these animals. We speculate that the relatively minor downregulation of SP-D leaves enough residual function of this protein in the animals to support baseline surfactant homeostasis. It is difficult to draw any major clinical implication from the small decrease in SP-D expression, but it can be speculated that it affects the lung function and is involved in the etiology of the mild signs of chronic inflammation.

The other surfactant proteins, SP-A, SP-B, and SP-C, are affected to an even lesser extent by ErbB4 deletion in these adult lungs. Since we have demonstrated that in vitro down-regulation of the ErbB4 receptor using siRNA downregulates surfactant synthesis in fetal type II epithelial cells (40, 41), we assume that compensatory mechanisms through other ErbB receptors must have taken place in vivo leading to the normal surfactant protein expression of SP-A, SP-B, and SP-C in these adult mice. Redirection of ErbB signaling toward formation of other ErbB receptor hetero- and homodimers may compensate parts of the missing function of ErbB4 in the developing surfactant system. It can be speculated that ErbB4 deletion leads to a shifting in dimer formation resulting in alveolar simplification seen in HER4null lungs. Indeed, growth factor stimulation leads to different biological results dependent on which ErbB receptor dimer partners are involved (32). Ligand binding by ErbB receptor stimulates homo- and/or heterodimer formation leading to their extensive signaling potential and signal diversification (25, 27). Redirection of ErbB1/ErbB4 signaling seen in type II cells under normal conditions (40) toward ErbB2/ErbB4 dimer formation in dexamethasone-exposed type II cells might play an important role in dexamethasone induced changes in the surfactant system of the type II epithelial cells (7).

ErbB receptors are known to be involved in lung development, injury, and remodeling. Deletion of ErbB1, also called EGFR, results in severe structural and functional changes in the lung, causing postnatal respiratory distress in these animals. The lungs of EGFR-deficient mice have impaired branching, a significant reduction of gas exchange surface, and surfactant deficiency (20, 29). EGFR forms heterodimers with ErbB4 and

The increased number of granulocytes and decrease in SP-D expression in ErbB4-deleted lungs confirm the presence of a chronic ongoing inflammatory process in those lungs. SP-D knockout mice show signs of postnatal inflammation (2, 17), a reduced number but an increased size of alveoli, which is combined with a decreased surface area of the alveoli (26, 38). These results are in agreement with our findings in the HER4null mice. However, we have not detected the additional described hypertrophy and hyperplasia of type II cells combined with giant lamellar bodies and the occurrence of foamy macrophages (17). Also, SP-D null mice have normal lungs at birth, and postnatal macrophage influx leads to an enlargement of the air spaces (2, 17), implying remodeling of normally developed alveoli in these animals. This observation leads us to the speculation that the minimal SP-D deficiency in

A

SP-A (26kDa)  
+/-  
-/-

SP-B (42kDa)  
+/-  
-/-

SP-C (26kDa)  
+/-  
-/-

SP-D (42kDa)  
+/-  
-/-

actin (42kDa)  
+/-  
-/-

B

![Intensity of bands (%)](image)

Fig. 4. Expression of surfactant proteins (SP) A, B, C, and D in control HER4heart+/− and HER4heart−/− lungs by Western blotting. A: mouse lung homogenates from control HER4heart+/− and HER4heart−/− animals were subjected to Western blotting to detect SP-A, SP-B, SP-C, and SP-D. α-Actin was used to control for equal protein loading. A representative blot for all surfactant proteins is shown. B: densitometric readings of blots from control HER4heart+/− and HER4heart−/− lungs. Intensity of surfactant protein bands was measured and normalized to the α-actin content of the sample. Expression of SP-D was downregulated in HER4heart−/− lungs. Data are shown as mean (%) ± SE. n = 11–12; *P < 0.05.

![Intensity of bands (%)](image)

Fig. 5. Expression of SP-A, SP-B, SP-C, and SP-D in control HER4heart+/− and HER4heart−/− lungs (by real-time PCR). The differences in the cycle threshold (DCt) of SP-A, SP-B, SP-C, and SP-D and actin genes were determined in both genotypes. Actin was used as an internal control to normalize the surfactant protein cDNA levels. The DCt values of the HER4heart+/− lungs were compared with DCt values from lungs of HER4heart−/− control animals (DDCt). DDCt values are inversely proportional to the levels of surfactant protein mRNA. Data are shown as means ± SE; n = 7–11; *P < 0.05.
is the most prominent dimer partner in the fetal lung (40). Some of the effects seen in our animals might be due to altered EGFR signaling. Protein expression of the other ErbB receptors is not significantly altered in these lungs (data not shown), and further studies of ErbB receptor interactions need to be carried out in isolated cell types. On the other hand, a decreased concentration of EGF is found in the bronchoalveolar lavage fluid of infants who develop respiratory distress syndrome (RDS) or BPD, implying an involvement of EGF and its receptor in the development of BPD (6). Inhibition of pulmonary ErbB2/ErbB3 signaling by downregulation of ErbB3 expression protects from the development of pulmonary fibrosis (21).

ErbB receptors are also known for their central role in a wide variety of biological processes (19). They contribute to the development of many forms of human cancer where overexpression of the receptor, mutations in the receptor gene, or an autocrine ligand production plays a role in the aberrant activation of these receptors. Interfering with these aberrant pathways opens avenues for the treatment of the resulting diseases, which is already in wide use of many tumors. This approach might be a therapeutic option for ErbB receptor dysregulation in the development of other diseases.

The number of infants with significant BPD has actually increased, secondary to improvements in neonatal survival. This is due to the development of new therapeutic strategies in obstetric and neonatal care. BPD appears to be initiated by a potent inflammatory response that develops in the lung secondary to prenatal infection, subsequent chorioamnionitis, and postnatal exposure to hyperoxia and mechanical ventilation (16) needed in infants with an insufficient surfactant system developing RDS. Exact signaling pathways in this scenario are unknown. Detailed knowledge about the prominent regulatory processes in the developing lung will provide needed cues for developing improved antenatal interventions to prevent RDS and BPD. Our findings suggest that this animal model might be helpful for further studies in the development of BPD.

In summary, our findings suggest that the ErbB4 receptor is required for the normal development of pulmonary alveoli leading to a normal lung function. We speculate that this transgenic ErbB4 mouse model is useful in gaining further insight in the pathogenesis of BPD. Our observations might help open avenues for prevention of neonatal lung disease that arises due to insults such as in inflammation or dexamethasone treatment.

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DISCLOSURES

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REFERENCES