Overexpression of TGF-α increases lung tissue hysteresivity in transgenic mice

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Pillow, Jane J., Thomas R. Korfhagen, Machiko Ikegami, and Peter D. Sly. Overexpression of TGF-α increases lung tissue hysteresivity in transgenic mice. J Appl Physiol 91: 2730–2734, 2001.—Increased transforming growth factor (TGF)-α has been observed in neonatal chronic lung disease. Lungs of transgenic mice that overexpress TGF-α develop enlarged air spaces and pulmonary fibrosis compared with wild-type mice. We hypothesized that these pathological changes may alter the mechanical coupling of viscous and elastic forces within lung parenchyma. Respiratory impedance was measured in open-chested, tracheostomized adult wild-type and TGF-α mice by using the forced oscillation technique (0.25–19.63 Hz) delivered by flexiVent (Screq, Montreal, PQ). Estimates of airway resistance (Raw), iner- tance (I), and the coefficients of tissue damping (Gt) and tissue elastance (Ht) were obtained by fitting a model to each impedance spectrum. Hysteresivity (η) was calculated as Gt/Ht. There was a significant increase in η (P < 0.01) and a trend to a decrease in Ht (P = 0.07) of TGF-α mice compared with the wild-type group. There was no significant change in Raw, I, or Gt. Structural abnormality present in the lungs of adult TGF-α mice alters viscoelastic coupling of the tissues, as evidenced by a change in η.

CHRONIC LUNG INJURY THAT DEVELOPS IN SOME NEONATES after premature birth is characterized by the persistence of simple, evenly distributed terminal air spaces, which are interspersed between evenly widened septa with hypercellular fibrous stroma and increased amounts of subepithelial elastic tissue with minimal evidence of airway disease (1). We hypothesized that the presence of structural lung disease that alters the amount of collagenous and elastic fibers may disrupt the normal mechanical balance between viscous and elastic forces within lung parenchyma. Development and clinical application of lung-function techniques that are able to detect viscoelastic properties of lung parenchyma could provide important insights into the evolution, course, and resolution of chronic lung disease in the neonate. As an initial step in evaluating the potential clinical merit of this hypothesis, this study aimed to determine whether viscoelasticity of lung parenchyma in adult mice with structurally abnormal lungs is different from that measured in age-matched healthy wild-type mice.

Transgenic mice overexpressing transforming growth factor (TGF)-α under the control of a surfactant protein C (SP-C) promoter gene were previously developed by Korfhagen et al. (10). TGF-α is a 50-amino acid polypeptide member of the epidermal growth factor family and has been found in respiratory epithelium, interstitial tissues, and alveolar macrophages within the lung. TGF-α is expressed in respiratory epithelium after severe neonatal lung injury and may contribute to the development of chronic lung disease in this population (20, 21). Increased expression of TGF-α has also been seen in rats with bleomycin-induced fibrosis (14a) and after asbestos exposure (11a) and associated with oxidant injury within the lung (23).

Hardie et al. (9) reported developmental changes in postnatal lung morphology and physiology of TGF-α transgenic mice, demonstrating abnormally large alveolar spaces and pulmonary fibrosis. When TGF-α mice were compared with wild-type mice, these structural changes were associated with an increase in specific compliance obtained from pressure-volume curves on excised lungs and evidence of abnormal lung function by measuring enhanced pause (Penh) (9). However, because Penh does not measure lung mechanics directly and is subject to influences such as changes in breathing patterns, the nature of the abnormality in lung function is not known. Both of these measurements, however, are crude reflections of structural changes in the lung. The current study used the low-frequency forced oscillation technique (FOT) to provide important new and detailed information about altered viscoelastic properties of the lung parenchyma in this model of structurally abnormal lung disease and compared these findings with respiratory mechanics deter-

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determined from single-frequency measurements of pressure and flow during tidal breathing.

MATERIALS AND METHODS

Mice. Seven adult female wild-type mice (strain FVB/N) and seven adult transgenic mice (SP-C-TGF-α) comprised of six males and one female (strain FVB/N) were used for the measurement of lung function as described below. Experiments were performed on the lungs of each mouse in accordance with the guidelines of the Australian National Health and Medical Research Council and with the approval of the Animal Ethics Committee of the Institute for Child Health Research.

Measurement of lung function. Mice were weighed and selected for lung function studies in a random order. Each mouse was anesthetized with 0.1 ml/10 g body wt of a mixture containing xylazine (0.4 mg/ml; Bayer) and ketamine (8 mg/ml; Parnell). Two-thirds of the dose was given to induce anesthesia; the remainder was given when the animal was attached to the ventilator. Top-up doses were given approximately each 40–60 min as required.

Once surgical anesthesia had been established, a tracheostomy was performed and a polyethylene cannula (1.0 cm; internal diameter = 0.23 mm) was inserted. Mice were ventilated with a tidal volume of 8 ml/kg at a rate of 450 breaths/min by using a custom-designed ventilator (flexiVent, Scireq, Montreal, PQ). Special features of this ventilator include a precision computer-controlled piston that is capable of accurately producing any desired waveform and accurate measurements of delivered volume (and thus flow) by tracking piston movement (with appropriate corrections for gas compression). A positive end-expiratory pressure (PEEP) of 0.2 kPa was established by fixing the tip of the expiratory limb 2 cm below the surface of a jar of water. A bilateral thoracotomy was performed to expose the pleural surface of each lung and to allow measurement of pulmonary impedance by excluding the contribution of the chest wall.

Multiple-frequency pulmonary mechanics. Baseline lung function was measured by using a modification of low-frequency FOT (8) above a PEEP of 0.2 kPa. Input impedance of the lung was measured between 0.5 and 19.6 Hz by applying a composite signal containing 19 mutually prime sinusoidal waves (2) with an amplitude of 1.6 ± 0.16 ml/kg during pauses in regular ventilation. The flexiVent ventilator was used for both regular ventilation and delivery of the oscillatory signal without the need to disturb the mice. The calibration procedure removes mechanical impedance of the tracheal cannula, and data reported represent mechanical properties on the lower pulmonary system alone.

A parameter-estimation model described by Hantos et al. (8) was used to partition lung impedance into components representing mechanical properties of the airways and parenchyma. This model consists of a frequency-independent airway resistance (Raw) and inertance (I) and a constant-phase tissue component [(GL - jHL)/ω²], where GL and HL are the coefficients for tissue damping and tissue elastance, respectively, ω is angular frequency, and α determines the frequency dependence of the real and imaginary parts of the impedance. All frequencies were included in the model fitting except those coinciding with heart rate and its harmonics. Tissue hysteresivity (η) (4) was calculated as the ratio of GL and HL.

Single-frequency pulmonary mechanics. Measurements of pulmonary mechanics were collected with a PEEP of 0.2 kPa and by using a 1-Hz sinusoidal forcing function. Measurements such as these are conventionally known as dynamic pulmonary mechanics and will be referred to as such in this manuscript. Dynamic resistance (RL,dyn) was derived from the relationship between airway opening pressure (Pao) and flow. Dynamic elastance (EL,dyn) was calculated from Pao and tidal volume.

Calculations and statistical analysis. Results are shown as means ± SE. Mechanical parameters were compared between wild-type and TGF-α mice by using Student’s t-test. Statistical significance was determined at P < 0.05.

RESULTS

There was no significant difference in mean body weight of the SP-C-TGF-α mice compared with the wild-type group (30.4 ± 2.7 vs. 34.1 ± 1.5 g; P = 0.25). All results are therefore expressed as absolute values.

Multiple-frequency pulmonary mechanics. A representative lung-impedance spectrum from each mouse group is shown in Fig. 1. Parameters were derived from application of the empirical model to the impedance spectra, obtained by using FOT, and are illustrated in Fig. 2. There was no significant difference between TGF-α and wild-type mice in Raw (P = 0.33), I (P = 0.66), GL (P = 0.51), or adequacy of the model fit. However, there was a trend for HL to be lower in TGF-α mice compared with wild-type mice (P = 0.07). η was higher in TGF-α mice; this last difference being highly significant (P < 0.01).

Single-frequency pulmonary mechanics. No significant differences were observed between TGF-α mice and wild-type mice for either RL,dyn (P = 0.39) or EL,dyn (P = 0.30), as shown in Fig. 3.
DISCUSSION

This study used a mouse model of pulmonary emphysema and fibrosis to investigate the potential application of low-frequency FOT as a noninvasive indicator of structural lung disease. The most notable findings included a highly significant, albeit small, rise in parenchymal $\eta$ of the TGF-$\alpha$ group compared with wild-type mice. The increase in $\eta$ resulted from an apparent decrease in $H_L$ in the absence of a significant change in $G_L$ in TGF-$\alpha$ mice compared with wild-type mice. Although hysteresis of the airways, tissues, or both has been previously noted to increase after bronchoconstrictor challenge (4, 6, 11–13, 19), there have only been isolated reports from whole lung preparations showing evidence of changes in $\eta$ with alterations to lung structure (3, 18, 22). Studies undertaken by Verbeek et al. (22) and Dolhnikoff et al. (3), however, used invasive techniques unsuitable for use in the clinical setting, whereas the recent study by Pillow et al. (18) used a noninvasive FOT to demonstrate a reduction in $\eta$ in the lungs of premature lambs exposed to multiple maternal antenatal steroid injections. The current study supports our hypothesis that structural abnormalities in the lung are associated with altered $\eta$ and suggests a potential role of the FOT in eliciting this information in a clinically acceptable manner.

The traditional paradigm that resistance and $H_L$ are respective mechanical properties of airway and parenchymal compartments of the respiratory system has been challenged by Fredberg and Stamenovic (4), who proposed that dissipative and elastic processes within the lung are intimately linked. By using previously published data from cats, humans, monkeys, and dogs, they observed that the ratio of lung tissue resistance to $H_L$ was a species-independent constant that they called $\eta$ of the lung (4). The highly conserved nature of this attribute suggests that $\eta$ may have an important role in the preservation of respiratory well being and that altered $\eta$ could reflect either structural or functional abnormalities.

The primary benefit of the current study over previous physiological assessments of TGF-$\alpha$ mice is the in vivo application of FOT to partition mechanical properties of lung tissues from the airways. The clear advantage of FOT compared with single-frequency lung function tests commonly utilized in the clinical setting is evident when comparing the sensitive detection of differences in $H_L$ and $\eta$ obtained by using FOT with the inability to detect significant differences in either $R_{L,\text{dyn}}$ or $E_{L,\text{dyn}}$ by using multiple linear regression analysis on single-frequency measurements. $R_{L,\text{dyn}}$ comprises elements of both airway and tissue resistance. As lower frequencies are heavily influenced by tissue properties, balance between these two elements will vary with the frequency at which the measurement is taken and may also be influenced by degree of lung maturation (18) and presence of lung disease. Many clinical studies are based on multiple linear regression analysis of single-frequency signals frequently imposed by a mechanical ventilator. Resultant calculated variables $R_{L,\text{dyn}}$ and $E_{L,\text{dyn}}$ represent resistive, viscoelastic, and elastic properties of the lung calculated at a single frequency. Multifrequency FOT measurements have the advantage of recording information at a number of frequencies simultaneously. This provides more comprehensive information about mechanical properties of the lung. The data allow the fitting of a four-parameter model that is capable of partitioning lung mechanics into variables representing airway and lung-tissue mechanics. In the present study, the application of FOT allowed for the noninvasive determination of $\eta$, which was found to be decreased in TGF-$\alpha$ mice compared with wild-type mice.

Fig. 2. Mechanical parameters of the lung in wild-type and TGF-$\alpha$ mice obtained by using FOT. Measurements in both groups were taken by using a positive end-expiratory pressure (PEEP) of 0.2 kPa. Raw, airway resistance; $I$, airway inertance; $G_L$, coefficient of tissue damping; $H_L$, coefficient of tissue elastance; $\eta$, hysteresivity. *$P < 0.01$. # $P = 0.07$.

Fig. 3. Dynamic resistance ($R_{L,\text{dyn}}$) and elastance ($E_{L,\text{dyn}}$) of wild-type and TGF-$\alpha$ mice. Measurements were performed at a PEEP of 0.2 kPa during tidal breathing.
study, the value of the more comprehensive assessment of lung function is clearly demonstrated. TGF-α mice have abnormal lung structure, yet this is not detected by the commonly used single-frequency measurements of $R_L$, $dyne$ and $E_L$, $dyne$. The more comprehensive assessment reveals changes in $H_L$, which just fail to reach statistical significance ($P = 0.07$), and a significant difference in $\eta$ ($P < 0.01$) between groups. The finding of a strong trend of reduction in $H_L$ that was not mirrored by similar changes in energy dissipation or $G_L$ supports earlier in vivo published evidence that the presence of structurally abnormal lung tissue causes altered mechanical coupling ($\eta$) of the lung tissue (3, 18).

By definition, any change in $\eta$ represents an altered balance between dissipation and storage of energy within the lung. Fredberg and Stamenovic (4) proposed four separate mechanisms that may contribute to altered dissipative behavior of the lungs, including ruptured cross bridges between actin and myosin, buckling of the surface-active film during deflation, stretching of the connective tissue network, and imperfect energy conservation because of recruitment and derecruitment of alveolar units.

There is good evidence that the connective tissue network is disrupted in transgenic TGF-α mice. Hardie et al. (9) showed that lungs of SP-C-TGF-α transgenic mice develop progressive structural abnormalities, including enlarged parenchymal airspaces and pulmonary fibrosis, associated with increased collagen deposition within the interstitium and on the pleural surface. Likewise, Korhagen et al. (10) observed that the elastin network is disrupted in these mice with shorter and blunter elastin fibers in the bronchiolar regions and abnormal alveoli with reduced secondary septation. Ganser et al. (5) showed that the treatment of mouse lung with TGF-α increases production of type IV collagenase and gelatinase.

It is clear that this altered parenchymal and airway architecture influences the mechanical behavior of the lung. The earlier observation by Hardie et al. (9) of an increased specific compliance in excised lungs of TGF-α mice compared with wild-type mice is supported by our observation of a trend of reduced $H_L$ in vivo. It is likely that the altered balance between collagenous and elastic components of lung parenchyma predisposes the lungs of TGF-α mice to overdistension and emphysema.

Although an alternative explanation for our results is that the increased $\eta$ is because of the presence of peripheral lung inhomogeneities, other investigators (14, 17) have shown that only a marked degree of inhomogeneity can add a virtual component to $G_L$ and $\eta$ and result in an altered frequency dependence of $H_L$. A consequence of these changes would include a compensatory decrease in $I$ in the model fitting, a result not observed in this study. Likewise, use of an input signal with mutually prime frequencies would have minimized the effects of nonlinearities on impedance data (2). Given the known deficient alveolar elastin network in these mice, our finding of increased $\eta$ is most consistent with either altered mechanical interactions between the entrance to alveolar ducts and the interstitium or stretching of the connective tissue network in TGF-α mice.

Alteration in the amount, function, or concentration of surfactant may alter the geometry of the alveolar surface and change $\eta$ of the lung tissue. Both alveolar surfactant pool sizes and alveolar surface area are decreased in TGF-α transgenic mice (unpublished observations). It is therefore likely that both surfactant concentration and function are normal, suggesting that the major factor accounting for increased $\eta$ is a result of structural change rather than altered surface-tension forces.

A clear difference between our study and the earlier study by Hardie et al. (9) is the difference in airway function. Whereas Hardie et al. concluded from Penh measurements that there was evidence of airway obstruction, we were unable to detect a significant change in Raw and if anything showed a trend in the opposite direction. Penh does not measure lung mechanics directly; instead, it infers a change in mechanical properties of the lungs from changes in the breathing pattern of conscious, unrestrained animals. Although there is an apparent correlation between changes in Penh and Raw during acute changes in lung mechanics (e.g., constrictor challenges) in animals with normal lungs (7), Penh can be affected by anything that alters an animal’s breathing pattern (15). A recent study by Peták et al. (16) demonstrates beyond doubt that Penh does not reflect lung mechanics in animals with abnormal lungs. They showed increased in Penh in mice with chronic exposure to hypoxia. In contrast, objective measurements of lung mechanics in the same mice showed increased lung stiffness with a reduction in Raw. We would contend, therefore, that our measurements of lung function more correctly reflect the changes in lung mechanics in this animal model than those observed previously by Hardie et al. (9).

**Clinical relevance.** Patients with structurally abnormal lungs account for a significant proportion of respiratory disease in the community. Development of minimally invasive technology that is sensitive to changes in the viscoelastic properties of lung tissue has the potential to significantly impact our understanding of the pathophysiological consequences of respiratory disease and may provide an index of structural integrity of the tissues. This study has shown that parenchymal $\eta$ is influenced by the development of emphysema and pulmonary fibrosis. Although the change in $\eta$ reported in the current study was small, it needs to be noted that affected mice had survived until adulthood. It is conceivable that the presence of more severe or acute disease may effect more dramatic changes in this parameter, which may provide a clinically useful measure of disease severity and response to treatment. FOT may be a useful adjunct in the assessment and monitoring of respiratory illness and the effects of treatment on respiratory function. Further measurements of $\eta$ both in animal models of structurally abnormal lungs and in humans with lung disease may
help to elucidate the potential role of this parameter in the clinical scenario.

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REFERENCES


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