Contribution of airway closure to chronic postbronchiolitis airway dysfunction in rats

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Asthma is a complex disease that often begins in childhood, probably as a result of interactions among genetic, environmental, and developmental factors that create an aberrant injury/inflammation/repair process in the airways (20). While airflow limitation and obstruction is the defining clinical feature of asthma, and a number of mechanisms have been identified that may contribute to this problem, our understanding remains limited regarding the airway locations and the predominant mechanisms of the airway obstruction of asthma. Increased airway closure during normal deflations from total lung capacity (TLC) or during challenges with methacholine has been reported in subjects having frequent exacerbations of asthma, compared with subjects having more stable asthma (8, 14), and it has been suggested that peripheral airway instability may be an important mechanism of obstruction in severe asthma (18, 29). A relevant animal model of asthmalike airway dysfunction would allow assessment of the importance and the mechanisms of airway closure.

After recovering from acute viral bronchiolitis, weaning Brown Norway (BN) rats develop a chronic asthmalike syndrome after recovering from viral bronchiolitis at an early age. We hypothesized that airway closure is an important mechanism of airflow obstruction in postbronchiolitis rats. Rats were studied 8–12 wk after inoculation with Sendai virus or sterile vehicle at 3–4 wk of age. Under light pentobarbital anesthesia, rats were instrumented with an orotracheal catheter and an esophageal pressure monitor and placed in a total body plethysmograph. Lung volumes and forced-expiratory maneuvers were measured using the Boyle’s law method and software-controlled valving of positive and negative pressures to elicit lung inflations and rapid deflations; pulmonary resistance was measured during spontaneous tidal breathing; and quasi-static pressure-volume curves were obtained with passive inflations and deflations in fully anesthetized, paralyzed rats. Compared with controls, the postbronchiolitis rats had elevated pulmonary resistance and reduced forced-expiratory volume in 0.2 s. Most of the reduced forced-expiratory volume in 0.2 s was associated with reduced forced vital capacity, indicating premature airway closure as a prominent mechanism. The reduced airflow in postbronchiolitis rats was highly dependent on lung volume, being nearly normal at 70% lung capacity, but sevenfold less than normal at 30% lung capacity. Increased respiratory system hysteresis between functional reserve capacity and total lung capacity was evidence for increased airway closure at normal end-expiratory lung volumes in postbronchiolitis rats. We conclude that airway instability and closure is a prominent mechanism of the chronic airway dysfunction in rats that have recovered from viral bronchiolitis at an early age.

METHODS

Animals. Male pathogen-free inbred BN/SnNHsd rats were purchased from Harlan (Indianapolis, IN) as 3-wk-old weanlings and housed in an American Association for Accreditation of Laboratory Animal Care-accredited isolation facility. All procedures involving animals were approved by the University of Wisconsin Animal Care and Use Committee.

Viral inoculation of rats. Rats were inoculated at 3–4 wk of age with parainfluenza type 1 (Sendai) virus (strain F3193) by exposing them to an aerosol containing 108 plaque-forming units of virus/ml, 2.6–2.7 ml of which were delivered into a Glas-Col Aerosol Exposure Apparatus (Glas-Col, Terre Haute, IN) over 20 min. Noninfected control rats were sham-inoculated with sterile vehicle (diluted chloroform, and Use Committee.

Institution for physiological studies. Physiological measurements were performed in lightly anesthetized (pentobarbital, 40–45 mg/kg ip; Abbott Laboratories, North Chicago, IL), spontaneously breathing rats that were 11–17 wk of age. An otracheal tube (PE-240, 5 cm) was placed atraumatically under direct visualization using a modified otoscope. Changes in pleural pressure were measured with a water-filled PE-160 esophageal catheter attached to a pressure transducer, and airway opening pressure was measured with an air-filled transducer attached to the tracheal tube connector. The esophageal catheter was positioned in the lower esophagus at a level having a distinct cardiac artifact and negligible differences between esophageal and airway opening pressure changes during an occluded inspiratory effort.

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Lung volumes and forced-expiratory maneuvers. Lung volumes and flow-volume loops were measured by using a total body plethysmograph equipped with software-operated solenoid valves connecting the tracheal catheter to outside air or to positive and negative pressure reservoirs (Buxco Electronics, Sharon, CT). The plethysmograph was modified with a copper wire heat sink, which minimized adiabatic compression artifacts. Intubated rats were placed in a right lateral recumbent position in the plethysmograph, and three lung inflations to 30-cmH₂O pressure were delivered before each set of measurements. The forced-expiration maneuver consisted of a positive pressure inflation from end-expiratory lung volume to TLC over 1.5–2 s, followed by a 1-s breath hold. TLC was defined as the lung volume at a plateau airway opening pressure of 30–32 cmH₂O after the 1-s breath hold; this plateau was obtained by adjusting the maximal pressure during inflation to 35-cmH₂O over 1.5–2 s, followed by a 1-s breath hold. TLC was defined as the lung volume at a plateau airway opening pressure of 30–32 cmH₂O after the 1-s breath hold; this plateau was then obtained by adjusting the maximal pressure during inflation to 35–37 cmH₂O. After the 1-s breath hold, the lungs were deflated rapidly to residual lung volume (RV) by using −40-cmH₂O pressure applied to the orotracheal tube. This deflation pressure gradient resulted in maximal expiratory flow rates at lung volumes <70% TLC, as indicated in separate studies of normal rats by similar flow rates at volumes ≤70% TLC with further increments in driving pressures to −70 cmH₂O. The resulting expiratory flow-volume loop was analogous to standard spirometry in human studies. The variables inspiratory capacity (IC), forced vital capacity (FVC), and expiratory reserve volume (ERV) were then calculated from the forced-expiratory volume in 1 s (FEV₁) in humans were determined for a minimum of two maneuvers, and an average from all acceptable maneuvers was recorded. The IC was defined as the change in lung volume from the end-expiratory volume during tidal breathing to the volume obtained after a 1-s breath hold at 30- to 32-cmH₂O inflation pressure. FEV₀.₂ was the change in lung volume during the first 0.2 s, and FVC was the total change in lung volume during the forced expiration, which was terminated when airflow became <0.5 ml/s. Maneuvers were excluded if there was an erratic flow-volume waveform (usually caused by secretions that were then removed), if the plateau airway opening pressure was outside the 30- to 32-cmH₂O range, or if a value for the IC or FVC varied from the other maneuvers by >5%. After completion of the forced-expiratory maneuvers, the plethysmograph was changed from a constant pressure to constant volume mode to increase sensitivity to small-volume changes. Following three lung inflations to 30-cmH₂O pressure and a return to normal tidal breathing, the airway opening was occluded at end-expiratory lung volume for 8 s while the changes in airway pressure and thoracic volume were recorded during inspiratory efforts. Functional residual capacity (FRC) was computed from these data and the ambient barometric pressure by the Boyle’s law method (Pulmonary Manoeuvres Software, Buxco Electronics). TLC was computed as TLC = FRC + IC, using the mean FRC from a minimum of two measurements.

Pulmonary resistance. Airflow resistance was measured in some of the rats after the forced-expiration maneuvers and the FRC measurement were completed. Anesthetized rats instrumented with an orotracheal tube and an esophageal pressure monitor were placed in a constant-pressure body plethysmograph in a right lateral position and allowed to breathe spontaneously to the outside. Transducer signals were fed to a pulmonary mechanics analyzer (model XA, Buxco Electronics, Sharon, CT), which integrated the flow signal to obtain tidal volume and computed resistance for each breath as an average over the entire breath. Resistance was recorded as an average over 1 min of spontaneous breathing, preceded 2 min by removal of secretions from the trachea and inflation of the lungs to TLC. The tracheal tube and connector resistance, adjusted for the mean airflow rate during the measurements in the individual rats, was subtracted from the measured resistance to determine pulmonary resistance, and specific pulmonary resistance (sRL) was computed as the product of pulmonary resistance and FRC to normalize for lung volume.

Quasi-static lung pressure-volume measurements. For the quasi-static lung pressure-volume measurements, the rats were fully anesthetized (pentobarbital, 60 mg/kg), paralyzed with succinycholine chloride (3 mg/kg ip; Sigma Chemical, St. Louis, MO), and ventilated mechanically before the maneuvers. These measurements were conducted in terminal studies of postbronchiolitis and control rats that were from different inoculation batches than those used for the other variables in this study, but that had similar postbronchiolitis pathology. The pressure-volume maneuver was performed with a syringe attached to the orotracheal tube, and airway opening pressure, esophageal pressure, and plethysmograph volume signals were recorded onto a computer using a WINDAQ/PRO* acquisition system (DATAQ Instruments, Akron, OH). The lungs were inflated twice to TLC and allowed to deflate passively to FRC for 10–15 s. The lungs were then inflated from FRC to 35-cmH₂O airway opening pressure over ~10 s, deflated over ~15 s to ~35 cmH₂O (RV), re-inflated from RV to 35 cmH₂O over ~15 s, and then deflated passively and returned to mechanical ventilation. The volume at 30 cmH₂O during the slow deflation from 35 cmH₂O was defined as TLC, and the TLC obtained from measures of IC and FRC before paralyzing the rat was then used to scale the volume axis. Pressure-volume curves were constructed as %TLC vs. airway opening pressure, and hysteresis was evaluated as the differences between isovolume pressure points at 60–80% TLC. We elected to use airway opening pressure rather than transpulmonary pressure because the change in esophageal pressures (representing the chest wall component during passive inflation and deflation) was small over the 60–80% TLC lung volume range, and, additionally, artifacts arising from esophageal tone, heartbeats, and mediastinal structures created substantial variability in the transpulmonary pressure measurements.

Data analysis. All volumes and flows are expressed in fractional units of TLC to normalize measurements to lung size. Data are presented as group means ± SD or as individual data points with group means. Comparisons of postbronchiolitis vs. control groups were conducted with the independent samples t-test for data conforming to parametric assumptions and, alternatively, with the Mann-Whitney test. Analysis of covariance, with body weight as the covariate, was used to test for group differences in lung volume relative to body size. Treatment group differences in expiratory flow rates and in respiratory system hysteresis were tested using treatment group and isovolume measurement points as categorical independent variables in a general linear ANOVA model, accounting for repeated measures in individual rats. All statistical tests were performed using SYSTAT version 10 software (SPSS, Chicago, IL).

RESULTS

Lung size and elasticity. TLC was linearly related to body weight in both groups of rats (Fig. 1), with the postbronchiolitis group averaging slightly lower lung volume relative to body mass compared with the control group (group difference P = 0.002, analysis of covariance). The upper limits of the deflation quasi-static pressure-volume curves were identical for the postbronchiolitis and control groups (Fig. 2), indicating that the respiratory system elastic recoil was not altered significantly from normal in the postbronchiolitis rats.

Airway obstruction. Specific pulmonary resistance, measured during quiet spontaneous breathing 2 min after a deep inflation, was elevated in the postbronchiolitis group (Fig. 3; P < 0.0001, Mann-Whitney test), indicating the presence of airflow obstruction. The FRC during spontaneous breathing also was elevated in the postbronchiolitis group (35 ± 3% TLC, n = 28) compared with the control group (30 ± 3% TLC, n = 36; P < 0.0001, t-test). The forced-expiratory maneuvers from TLC revealed reduced FEV₀.₂ in the postbronchiolitis rats (Fig. 4; P < 0.0001, t-test), indicating that airway obstruction was present during a deep inflation maneuver, as well as under
the conditions of quiet tidal breathing measured with sR L. Because FEV0.2 may be reduced by either premature airway closure or airflow limitation, we evaluated FVC as an indicator of airway closure and the FEV0.2-to-FVC ratio (FEV0.2/FVC) as an indicator of airflow limitation during a forced-expiratory maneuver. In the postbronchiolitis rats, the FVC was reduced by an average volume of 10% TLC compared with controls (Fig. 4; \( P < 0.0001 \), t-test), indicating that premature airway closure accounted for most of the observed reduction of FEV0.2 in these rats. In contrast, the average FEV0.2/FVC was only 3% lower in the postbronchiolitis rats compared with the controls (Fig. 4; \( P < 0.006 \)), suggesting that only minor airflow limitation was present over the lung volume range that was exhaled in the first 0.2 s. Reasoning that the FEV0.2/FVC might be insensitive to airway obstruction occurring primarily at lower lung volumes, we investigated whether there were lung volume-dependent changes in abnormal airflow in the postbronchiolitis rats. Figure 5A illustrates that maximal airflow, measured at isovolume points in the 30–70% TLC range during forced expiration, was reduced in the postbronchiolitis rats throughout this range of lung volumes (\( P < 0.0001 \), ANOVA). However, the maximal flow rates of the postbronchiolitis rats became progressively more reduced relative to those of normal controls as lung volume decreased from 70% TLC to 30% TLC (Fig. 5B), suggesting marked airway instability at lower lung volumes.

Given that both pulmonary resistance and forced-expiratory airflow were abnormal in the postbronchiolitis rats at the 30–40% TLC lung volume where tidal breathing occurs and that air-trapping occurred during forced expiration, we hypothesized that airway closure might be a significant component of airway obstruction during normal tidal breathing in these rats. We evaluated respiratory system pressure-volume hysteresis as a test of this hypothesis, reasoning that airway closure would increase the force required to inflate the lungs from FRC, resulting in increased hysteresis in the FRC-TLC-FRC loop. The postbronchiolitis rats exhibited greater hysteresis, due to a displacement of the inflation limb to higher pressures during inflation from FRC (Fig. 6A; \( P < 0.0001 \), ANOVA), consistent with the hypothesis that this group had increased airway closure at FRC. In contrast, during inflation from RV, when both groups would be expected to have extensive airway closure, the postbronchiolitis rats exhibited less hysteresis compared with the controls (Fig. 6B; \( P < 0.0001 \), ANOVA), suggesting that relatively less force was necessary to reopen closed lung units in the postbronchiolitis rats.
**DISCUSSION**

The results of these studies indicate that airway closure is a prominent component of the chronic airway dysfunction that develops in BN rats after recovery from viral bronchiolitis at an early age. The airway closure was detected directly as air trapping, with the FVC of the postbronchiolitis group being reduced an average volume of 10% TLC compared with that of the control group (Fig. 4). The postbronchiolitis rats also exhibited greater hysteresis in the respiratory system quasi-static pressure-volume curve measured between FRC and TLC, caused by increased isovolume pressures on the inflation limb of the curve. This increased hysteresis is evidence for increased derecruitment of lung units at FRC (5, 9), which could contribute to the elevated sRu. that was measured in the postbronchiolitis group during normal tidal breathing. Although derecruitment of lung units could occur as either alveolar collapse or airway closure, the elevated thoracic gas volume after maximal expiration indicates that airway closure (with trapping of gas in the alveoli) is an important mechanism of abnormal derecruitment of lung units in the postbronchiolitis rats. It is possible that airway closure and derecruitment of lung units is the sole mechanism operating to cause the airway dysfunction in postbronchiolitis rats, consistent with the model proposed by Anafi and Wilson (1) that describes two stable states, open and closed, for terminal airways. However, the data are consistent with a component of airflow limitation that could be due to reduced airway caliber, and the measured airway obstruction is a continuum of airway narrowing and closure. The FEV$_{0.2}$/FVC would be expected to detect airflow limitation within the vital capacity range, and the postbronchiolitis rats did exhibit significantly lower FEV$_{0.2}$/FVC in this study (Fig. 4), although these differences were quantitatively small compared with the treatment group differences in FVC. However, it is possible that, when FVC is reduced due to premature closure, airflow limitation may be more severe than the degree detected by FEV$_{0.2}$/FVC, because the fraction expired during the first 0.2 s occurs at a higher absolute lung volume. Evaluation of forced airflow rates at specific lung volumes revealed that the postbronchiolitis rats had lung volume-dependent airflow limitation that was only slightly different from controls at 70% TLC, but that diverged from normal during lung deflation, having a sevenfold reduction in mean flow rates compared with controls at 30% TLC (Fig. 5). If maximal airflow is determined primarily by lung elastic recoil and airway upstream conductance (25, 28), and if we assume that lung elastic recoil was similar at isovolume points for the two treatment groups (based on similar quasi-static deflation pressure-volume curves), then the progressive diversion in relative rates of airflow during expiration (Fig. 5) represents an accelerated reduction in airway conductance during deflation in the postbronchiolitis rats. The marked volume dependency of the airway obstruction is more suggestive of a distal, dynamic process than of a central, fixed obstruction, in that the smaller airways generally are more compliant and have relatively greater changes in diameter with changes in lung volume compared with central airways (2, 3).

Questions remaining to be investigated include the following: where in the airways does the excessive narrowing and closure occurs, and what mechanisms are responsible for premature airway closure in the postbronchiolitis rats? Closure in normal airways is thought to occur primarily by the formation of liquid menisci, which form at a critical airway diameter relative to the volume of fluid lining the airway lumen (16, 23) and thus could be enhanced by processes that either increase luminal liquid or reduce luminal diameter. Airways also can close by mucosal collapse or compression; this may occur only at negative luminal pressures, unless conditions exist at normal transpulmonary pressures that allow the forces favoring closure (luminal surface tension and airway wall smooth muscle tone) to exceed the forces favoring patency (lung elastic recoil and airway wall elastance) (10, 11, 13, 26). In the present study, we used hysteresis as an indicator of the forces needed to recruit lung units during inflation, and the increased hysteresis during inflation from FRC to TLC in the postbronchiolitis rats was taken as evidence for increased derecruitment at FRC, compared with control rats. However, when hysteresis was compared during lung inflation from RV, a volume at which extensive airway closure would be expected in both study groups, it was the control group that required relatively greater forces to recruit lung units during inflation (Fig. 6). The most likely explanation for this difference between postbronchiolitis and control groups is that derecruitment occurred in a different location in...
the postbronchiolitis lungs, for example, with closure occurring in a different population of airways, or with a change in the relative proportions of airway closure vs. alveolar collapse during derecruitment of lung units. Naureckas and colleagues (26) demonstrated in rat lungs that the reopening pressure for collapsed airways varied inversely with airway size; if the airway closure in postbronchiolitis rats occurred in larger airways than in control rats, this could explain the difference in hysteresis from RV in the two groups. This concept is consistent with imaging studies in humans that showed airway closure in persons with asthma having a different distribution, and possibly involving larger airways, compared with the airway closure patterns in nonasthmatic subjects (17). Alternatively, the airway wall thickening that is present in postbronchiolitis rats (35) may provide an increased elastic force within the airway wall (24) that could reduce the transbronchial force necessary to reopen a collapsed airway. There are a number of factors associated with airway inflammation that may contribute to premature airway closure by altering luminal fluid volumes and surface tension, airway wall thickness and elastance, smooth muscle activity, and airway-parenchymal coupling (18), and these may be collectively responsible for much of the airway instability in human asthma and in the postbronchiolitis rats. However, viral airway injury at an early age also may induce chronic airway instability, with elevated RV in rats.

Fig. 5. A: maximal airflow (group mean ± SD, in units of TLC volumes/s) at isovolume points, measured in control (n = 36) and postbronchiolitis (n = 28) rats at 8 wk postinoculation. B: relative maximal airflow rates of postbronchiolitis rats compared with controls, expressed as the postbronchiolitis group-to-control group ratio of the group means at each isovolume point.

Fig. 6. Airway opening pressure (Pao; means ± SD) hysteresis at 60–80% TLC, measured at 12 wk after inoculation during inflation from FRC (A) in control (n = 8) and postbronchiolitis (n = 14) rats, and during inflation from residual lung volume (B).
having no apparent chronic inflammatory process or changes in lung morphometry (34), suggesting that there are intrinsic factors affecting airway stability after recovery from bronchiolitis, in addition to those associated with chronic inflammation.

The airflow limitation and airway closure observed in this study were mostly in the context of a forced deflation from TLC, and thus the potential influence of a deep lung inflation on airway obstruction is relevant to the interpretation of the data. In asthmatic subjects, a single deep breath may cause a transient change in airway caliber, measured as a decrease in specific airway conductance and anatomic dead space, and as an increase in pulmonary resistance during the first 30 s after the inflation (4, 7). In contrast, no significant changes in airway conductance occur in nonasthmatic subjects in the first minute after a deep breath (7, 22), although, in normal subjects, a small reduction in forced-expiratory flow rates may be detected if the forced expiration is preceded by a pause of several seconds at TLC (6). Thus it is possible that differences between the postbronchiolitis and control rats with regard to the forced-expiratory maneuvers and the lung hysteresis may have been amplified by group differences in the effects of deep inflation on lung mechanics. However, it is unlikely that the airflow limitation observed in the postbronchiolitis group was entirely induced by the deep inflation maneuver, in that this group also had elevated pulmonary resistance under conditions of normal tidal breathing. Pulmonary resistance was measured 2–3 min after a lung inflation to mimic the volume history of normal, awake rats, which have spontaneous deep breaths about every 2 min (12), and to avoid the altered airway responsiveness that is associated with abstinence from deep breaths (22, 32). Although pulmonary resistance measured during spontaneous ventilation does not permit assessment of the relative contributions of airway narrowing and closure, the tissue component, heterogeneous airway narrowing, and airway wall shunting (21), the considerable difference in measured resistances between the two groups of rats that were breathing at similar frequencies is consistent with the airflow limitation detected with the forced-expiratory maneuvers.

Studies of human subjects with asthma indicate that airway closure may also be an important component of airway obstruction in this disease. Compared with subjects having normal airways, stable asthmatic subjects exhibited airway closure on SPECT/Technegas imaging in a patchy, uneven distribution that suggested involvement of a heterogeneous process separate from normal gradients in lung elastic recoil (17). Similarly, subjects with moderate to severe asthma were found to have ventilation defects, detected with magnetic resonance scanning after inhalation of hyperpolarized helium-3 gas, that were inversely related to FEV1 and worsened after methacholine or exercise challenges (30). Luchten and colleagues (22) utilized measures of pulmonary resistance and elastance over input frequencies of 0.1–8 Hz to identify heterogeneous airway narrowing and closure in asthmatic subjects with severe disease and in both asthmatic and control subjects after methacholine challenge. Other studies comparing the responses of subjects with normal vs. asthmatic airways found that challenges with methacholine or dry air hyperpnea increased RV in the asthmatic subjects, indicating probable airway closure and air trapping during these perturbations (15, 27). Gibbons and colleagues (8) evaluated responses to methacholine challenges in patients referred for bronchoprovocation testing and computed the change in FVC that was associated with a 20% drop in FEV1, to quantify the relative contributions of airflow limitation vs. airway closure to the measured response. Within this group of patients, there was a spectrum of responses ranging from negligible change in FVC to substantial changes in FVC that fully accounted for the 20% drop in FEV1. A retrospective review of oral prednisone prescriptions revealed that systemic steroid use was relatively greater for the patients who had the larger changes in FVC during methacholine challenge, suggesting that airway closure might be a feature of unstable asthma (8). Supporting this conclusion was a study showing increased closing capacities in currently stable asthmatic subjects who had a history of frequent exacerbations, compared with a well-matched group of asthmatic subjects with more stable disease (14). Airway closure thus is a component of the airway obstruction of asthma and is more prominent in unstable asthma.

In conclusion, we have identified airway closure as an important component of chronic postbronchiolitis airway dysfunction in rats. This animal model has a number of parallels with human asthma, and it is proving valuable for the understanding of mechanisms related to chronic airway diseases.

**REFERENCES**


