Compliance and stability of the bronchial wall in a model of allergen-induced lung inflammation

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ASTHMA IS COMMONLY associated with bronchial inflammation and is characterized by airflow limitation. The cause(s) of flow limitation is unclear, but it may involve changes in the mechanical properties of the lung. For example, changes in elastic forces in the lung could alter airway narrowing brought about either by active airway smooth muscle contraction or by compressive forces. Active airway narrowing is increased when the load on the airway from parenchyma is reduced (7). Macklem (18) has suggested that inflammation might reduce elastic load on the airway from lung parenchyma, thus facilitating bronchoconstriction.

Inflammatory changes could also alter load on the smooth muscle imposed from the airway wall itself (2). To our knowledge, elastic forces associated with the airway wall have not been directly or systematically examined in asthmatic patients or in animal models of allergic lung disease. There is evidence that an action of exogenous proteases on the airway wall may reduce airway compliance; airway stability; asthma; airway narrowing.

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Macklem, H. W., D. J. Turner, P. R. Gray, and P. K. McFawn. Compliance and stability of the bronchial wall in a model of allergen-induced lung inflammation. J. Appl. Physiol. 86(3): 932–937, 1999.—Airway wall remodeling in response to inflammation might alter load on airway smooth muscle and/or change airway wall stability. We therefore determined airway wall compliance and closing pressures in an animal model. Weaning pigs were sensitized to ovalbumin (OVA; ip and sc, n = 6) and were subsequently challenged three times with OVA aerosol. Control pigs received 0.9% NaCl (n = 4) in place of OVA aerosol. Bronchoconstriction in vivo was assessed from lung resistance and dynamic compliance. Semistatic airway compliance was recorded ex vivo in isolated segments of bronchus, after the final OVA aerosol or 0.9% NaCl challenge. Internally or externally applied pressure needed to close bronchial segments was determined in the absence or presence of carbachol (1 µM). Sensitized pig lungs exhibited immediate bronchoconstriction to OVA aerosol and also peribronchial accumulations of monocytes and granulocytes. Compliance was reduced in sensitized bronchi in vitro (P < 0.01), and closing pressures were increased (P < 0.05). In the presence of carbachol, closing pressures of control and sensitized bronchi were not different. We conclude that sensitization and/or inflammation increases airway load and airway stability.

Methods

Sensitization. Ten 4-wk-old female pigs (~10 kg) were purchased from a commercial piggery. Each pig was masked down with halothane (Fluothane, ICI), 4%. Six animals received a priming dose on day 1 of 4 mg OVA (Sigma Chemical) dissolved in 0.9% NaCl containing 64 mg Al(OH)3 as an adjuvant (ip and sc over 4 sites). On day 7, a booster dose of 1 mg OVA dissolved in 0.9% NaCl containing 64 mg Al(OH)3 was administered (sc), divided over four sites. Four control animals received injections of 0.9% NaCl on days 1 and 7.

Aerosol antigen challenge. Starting on day 14, three OVA aerosol challenges were administered at 3-day intervals. Pigs were first sedated with tiletamine/zolazepam (Zoletil 100, Virbac, 4.4 mg/kg im) and xylazine (Ilium Xylazil 100, Troy Laboratories, 2.2 mg/kg). Anesthesia was induced and maintained with methohexitone sodium (Brietal Sodium, Eli Lilly, 20 mg iv every 5 min). Pigs were intubated and paralyzed with pancuronium bromide (David Bull Laboratories, 0.2 mg/kg iv) and ventilated (Harvard Apparatus model 708) to maintain ventilation at the preparalysis level.

OVA aerosol (50 mg/ml in 0.9% NaCl) was generated by using a disposable nebulizer (Aerflo 1636, Waite) and oxygen as the carrier gas. Pigs were hand ventilated and breathed the aerosol for 5 min. In recovery studies OVA exposure was carried out under atropine (0.04 mg/kg im) and terbutaline (0.005 mg/kg sc) cover, and paralysis was reversed with 0.5 mg neostigmine (Astra Pharmaceuticals), with a further 0.02 mg/kg of intravenous atropine. In nonrecovery studies (see Tissue collection), atropine, terbutaline, and neostigmine were not given. Control animals were treated in an identical manner, except that the aerosol was 0.9% NaCl. Sensitization was checked by skin-prick test after day 14 (OVA 1.0 mg/ml, 50 µl id). A purple wheal of =2-mm diameter was deemed positive.
Pulmonary function. Total lung resistance (R_L) and dynamic lung compliance (Cdyn) were recorded before and immediately after the third OVA aerosol challenge, or 0.9% NaCl challenge in control animals. A pneumotachograph (Hans Rudolph 3500 series, 0–35 liters) and pressure transducer (Mumened) were connected to the end of the endotracheal tube to give a flow signal. Flow was integrated to give tidal volume. An air-filled esophageal cannula was positioned with the distal end in the midthoracic esophagus and the proximal end connected to a pressure transducer (Mumened) to measure transpulmonary pressure. Pressure, tidal volume, and airflow were used to calculate R_L and Cdyn during tidal breathing by using a pulmonary function computer (Mumened). R_L was calculated at points of equal lung volume during a breath, and Cdyn was calculated at zero flow.

Tissue collection. After completing the final OVA aerosol challenge, pigs were exsanguinated while still anesthetized. The lungs were removed and bronchial segments, some 2.5 cm in length, were dissected free of parenchyma, and the side branches were tightly ligated with surgical silk threads (19, 20). Segments were obtained from each of the upper and lower lung lobes (i.e., 4 segments from each pig). Segments were cannulated and mounted horizontally in organ baths containing a Krebs solution at 37°C and bubbled with 5% CO₂ in O₂ (Fig. 1).

The anatomic location (generation) of the proximal and distal ends of bronchial segments was determined from the numbers of side branches, with the trachea designated as generation 0. The proximal and distal internal diameters were determined by using machined metal rods gently inserted into the airway opening before cannulation. The volume of bronchial segments at 0 cmH₂O transmural pressure (Vi) was measured from the volume of Krebs solution required to fill the lumen, using the mean of three measurements in each segment. A comparison between this and alternative methods of recording airway volume has been critically reviewed (19).

Pressure-volume curves. Semistatic pressure-volume curves were generated by changing the luminal volume with a microsyringe (Fig. 1), in at least eight 0.02-ml increments (Fig. 2). Luminal pressure was recorded after >80% of the stress relaxation had occurred (2–3 min). Two complete inflation-deflation cycles were performed on each bronchus to standardize volume history before a third cycle was recorded that was used for analysis. Volume was expressed as strain by normalizing for Vi of the airway (i.e., ΔV/Vi), where ΔV is change in volume (1, 19).

Closing pressures. We recorded the intraluminal (internal) and adventitial (external) airway pressure that produced airway luminal closure (20). The negative intraluminal pressure required to close bronchial segments was assessed by reducing intraluminal volume in small increments (0.02 ml) at the distal end of the segment while pressure at the proximal end was recorded (Fig. 1). When the bronchial segment between the distal and proximal end closed, the measured pressure no longer changed with further reductions in volume. The pressure at which this occurred was defined as the internal closing pressure.
The compressive force required to close bronchi was determined manometrically, after a pressure-tight lid to the organ bath chamber was secured, with the distal end of the bronchus open to atmosphere (Fig. 1). Until compression occluded the bronchial segment, intraluminal pressure (recorded at the proximal end) remained at 1 atmosphere. Once the lumen closed, however, the intraluminal pressure increased with increased adversal pressure. The adversal pressure at which this occurred, recorded from the manometer, was defined as the external closing pressure. Bronchi were then allowed to recover at 0 cmH\(_2\)O transmural pressure before being contracted with 1 μM carbachol, and the external closing pressure was redetermined with the tissue contracted.

Histological assessment. Lobes were fixed in 10% Formalin for subsequent histological examination. Fixed tissues were paraffin embedded, then 5-µm sections were cut and stained with hematoxylin and eosin. Coded samples were assessed for the presence of lymphocytes, eosinophils, polymorphonuclear leukocytes, and macrophages in alveoli, peribronchial regions, and in the airway wall.

Statistics. We examined 10 upper lobe bronchi and 12 lower lobe bronchi from 6 sensitized pigs. From four control pigs, eight upper lobe and eight lower lobe bronchi were studied. Results are means ± SE, except for airway generation (Table 1), where the model is given. For in vivo studies, n is the number of animals; for in vitro experiments, n is the number of bronchi. In vivo responses to OVA (R\(_l\) and C\(_{dyn}\)) in test and control pigs were compared by using Student’s t-test. Anatomic details of airways from test and control pigs (Table 1) were examined by one-way ANOVA with Bonferroni’s post hoc test.

Compliance was calculated from the linear region of the pressure-volume (strain) curve, which was typically within the range of 15 to 20 cmH\(_2\)O, using simple two-stage regression analysis as fully described (19). Two-way ANOVA was used to test an effect of sensitization and airway location on specific compliance and closing pressure, and to establish whether there was interaction between airway location and sensitization. A paired t-test was used to study an effect of carbachol on closing pressures.

RESULTS

All sensitized pigs showed positive skin responses (wheals) within 5 min of OVA, whereas control pigs did not. Baseline R\(_l\) was 23.2 ± 2.0 and 24.0 ± 2.6 cmH\(_2\)O·l\(^{-1}\)·s\(^{-1}\) in control and sensitized pigs, respectively. Baseline C\(_{dyn}\) was 9.0 ± 1.2 and 10.2 ± 0.3 ml/cmH\(_2\)O, respectively. OVA (50 mg/ml for 5 min) increased R\(_l\) to 176 ± 25% of baseline and decreased C\(_{dyn}\) to 51 ± 13% (n = 6, P < 0.001). In control pigs R\(_l\) and C\(_{dyn}\) were 106 ± 3 and 95 ± 13% (n = 4) of baseline, respectively, after 0.9% NaCl aerosol. Histological studies demonstrated an accumulation of lymphocytes, macrophages, eosinophils, and neutrophils primarily in peribronchial and alveolar regions of sensitized but not control pigs.

The size (internal diameters and luminal volume) and generation of the bronchi used are shown in Table 1. There was no difference in airway generation, size, or luminal volume between bronchi from OVA-sensitized test pigs and control pigs.

Representative compliance curves for a sensitized and control upper lobe bronchi are shown in Fig. 2. Upper lobe bronchi were more compliant (P < 0.01) than lower lobe bronchi (Fig. 3). OVA sensitization significantly decreased bronchial compliance (P < 0.01, Figs. 2 and 3). Sensitization affected upper and lower lobe bronchi, and there was no interaction between airway location and effect of sensitization.

Negative intraluminal pressures closed upper (n = 8) and lower (n = 8) lobe control bronchi at 23 ± 6.0 and 17 ± 2.6 cmH\(_2\)O, respectively (P < 0.05). Significantly greater transmural pressures were required to close sensitized bronchi compared with bronchi in control pigs (P < 0.05, n = 10 upper and 12 lower lobe bronchi from sensitized pigs), with internal closing pressures increasing to 39 ± 9.0 and 25 ± 3.0 cmH\(_2\)O, respectively. There was no interaction between an effect of sensitization and airway location.

External closing pressures for upper and lower lobe bronchi were also increased by sensitization (P < 0.01, Fig. 4). However, unlike internal closing pressures, there were no significant differences in external closing pressure between upper and lower lobe bronchi (Fig. 4).

Carbachol (1 μM) increased active pressure by 30–40 cmH\(_2\)O in upper or lower lobe bronchi, and there were no significant differences between sensitized and control bronchi. Epinephrine (10 μM) had no effect on luminal pressure in either group of bronchi. Carbachol increased external closing pressure by 7.6 ± 1.2 cmH\(_2\)O in upper (P < 0.01, n = 8) and 13 ± 2.5 cmH\(_2\)O in lower lobe (P < 0.01, n = 8) bronchi from control pigs. Bronchi from sensitized pigs also showed an increase in external closing pressure after contraction, 6.7 ± 1.4 and 7.6 ± 1.7 cmH\(_2\)O in upper (n = 10) and lower lobe (n = 12) bronchi, respectively (P < 0.01). However, in sensitized bronchi the percent increase in external closing pressure after contraction was significantly less than for control bronchi (P < 0.05). After contraction there was no difference in closing pressure between sensitized and control bronchi.

| Table 1. Location and size of bronchial segments used to measure airway compliance and closing pressures |
|-----------------|------------------|------------------|------------------|------------------|
|                | Distal Internal Diameters, mm | Proximal Internal Diameters, mm | Proximal Generation | Distal Generation | Lumen Volume, ml |
| Control upper lobe | 2.88 ± 0.05 | 2.0 ± 0.0 | 5 | 11 | 0.058 ± 0.004 |
| Sensitized upper lobe | 2.88 ± 0.04 | 2.0 ± 0.0 | 5 | 11 | 0.054 ± 0.003 |
| Control lower lobe | 2.97 ± 0.04 | 2.05 ± 0.03 | 14 | 20 | 0.036 ± 0.003 |
| Sensitized lower lobe | 2.91 ± 0.04 | 2.0 ± 0.0 | 13 | 20 | 0.034 ± 0.002 |

Values are means ± SE, n. No. of bronchi. Bronchial segments were taken from the upper and lower lobes of pig lung. Sensitized pigs were sensitized to ovalbumin. Generation was counted, taking the trachea as generation 0. Luminal volume is the resting internal volume of the bronchial segments with 0 transmural pressure difference. There were no significance differences between sensitized and control bronchi.
DISCUSSION

This study is the first to investigate the effects of sensitization and inflammation on the pressure-volume relationship of the intact airway wall or on airway stability. Exposure to OVA produced inflammation of the lung and an immediate allergic reaction, as demonstrated by skin sensitivity and bronchospasm in response to OVA delivered by aerosol. The inflammation was characterized by a mononuclear infiltrate, with eosinophils and polymorphonuclear leukocytes, as reported previously with ascaris antigen (10). Our controls were not exposed to OVA (or adjuvant) at any stage of the experiment to avoid possible alterations in airway physiology as a result of specific antibody or
other serum factors (9, 24, 40). However, we have previously found that airway compliance is identical in control animals either immunized with OVA and with Al(OH)₃ as an adjuvant and subsequently given 0.9% NaCl aerosol challenge, or given 0.9% NaCl sham immunization and aerosol challenge as used in the present study (unpublished observations). Pressure-volume curves show that static compliance is decreased in whole lungs in asthmatic patients (6), but the compliance of the airway wall is unknown. In the present study we show decreased bronchial compliance in an animal model and an increase in transmural pressure needed to cause airway closure.

Airway compliance contributes to both pre- and afterloads on airway smooth muscle and is, therefore, a factor in airway function (14, 32, 33, 36). Unloaded smooth muscle can shorten by up to 80% of its resting length when it is maximally stimulated (36), yet loaded smooth muscle in situ shortens by ~40% (16, 21, 30). Airway compliance defines elastic properties of the combined airway wall, but that particular airway structures are most important to smooth muscle load is unclear and was not investigated in the present study. The changes in the mechanical properties of the airway wall in sensitized animals could arise from changes in wall morphology and/or collagen and elastin. In large airways, the cartilage (17, 39) and loose connective tissue in the outer airway wall (19, 22, 31) could be important to smooth muscle shortening or airway narrowing. In vivo, cell infiltration or edema might influence compliance, but in the present study there was no marked cell influx to the airway wall, and edema may be a lesser factor in the isolated preparation used by us than in vascularly perfused airway in vivo.

Is decreased compliance in the present study compatible with the physiological behavior of airways in asthmatic patients? Macklem (18) and others (26) have suggested that a reduced load in asthma could increase active muscle shortening and airway narrowing. However, our findings are not concordant with a hypothesis that airway load is decreased in inflammatory disease but rather provide a first indication that load can be increased. However, a stiff airway wall would be less susceptible to forces of interdependence from the parenchyma, which normally limit airway narrowing (7, 33). In normal subjects, a deep inspiration causes bronchodilation and/or reduces agonist-induced bronchoconstriction, possibly by stretching the airway smooth muscle, but in asthmatic patients it does not (29, 35). As suggested by others, a stiffer airway wall in asthmatic patients could account for the attenuated effect of inspiration on luminal caliber (6, 35). The present findings provide a possible basis for this proposal.

Elastic properties of the airway wall were also investigated by using airway stability. A role of airway collapse in flow reduction is uncertain in lung disease (38). However, our principal purpose was to determine whether changes to compliance were reflected in the wall stability, so we hypothesized that decreased compliance would increase the pressures needed to produce airway collapse or closure. Airway smooth muscle is located inside the cartilage, so we determined internal and external closing pressures in case there was interaction between wall structure and the direction from which force was applied. However, internal and external closing pressures were similar, and each was increased in sensitized animals, indicating that the structures affected by sensitization were accessible to forces from either direction.

Airways from widely different regions of the lung (from left and right upper and lower lobes) showed comparable mechanical changes with sensitization. Bronchi from the upper lobes were more compliant than those from the lower lobe but had greater internal closing pressures. External closing pressures were the same, however. There is no information about the main site(s) of airway resistance in pigs. However, intermediate-sized airways are a site of bronchoconstriction in this and other large species (3, 11, 23, 28). We have no information on changes to more peripheral or central airways in this model. However, the similar effects of sensitization in upper and lower lobes argue for uniformity in the response of intermediate-sized airways (e.g., 2 mm inner diameter) to the sensitization protocol.

The effect of carbachol on airway stability was determined to investigate a role of airway smooth muscle in the elastic properties studied. As partly confirmed in the present study, muscle contraction decreases airway compliance and increases stability (13, 20, 31) and may produce a paradoxical increase in airway diameter in compressed airways (5, 20). However, we found that smooth muscle contraction exerted less protection against airway closure in sensitized than control bronchi; in the presence of carbachol there were then no significant differences in closing pressure between the two groups. This was not explained by a presence of preexisting muscle tone in sensitized bronchi nor by a lesser contractile force to carbachol in sensitized bronchi. We conclude that contraction produced less stiffening in the sensitized group. An inference is that the mechanism producing the changes to airway compliance or stability does not primarily involve the smooth muscle. Contracted smooth muscle, by virtue of its increased stiffness, might become the major load-bearing structure in the airway wall, masking changes in the stiffness of other wall components brought about by sensitization. Furthermore, any rearrangement or tensioning of cartilage pieces by muscle contraction might also obscure more subtle changes in structural properties elsewhere in the airway wall that might be targets for remodeling in response to inflammation.

In summary, lung inflammation in this model increases airway stiffness and, by implication, airway wall loads on the smooth muscle. This argues against the hypothesis that remodeling during asthma may reduce the afterloads applied to the airway smooth muscle by the wall. Increased wall stiffness could, however, uncouple the airway smooth muscle from the lung parenchyma, reducing interdependence and contributing to exaggerated airway narrowing seen in...
asthma. The mechanisms by which inflammation increases stiffness are unknown, but evidence suggests that they do not involve the airway smooth muscle.

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