

Airway Hyperresponsiveness: From Molecules to Bedside

Selected Contribution: How does airway inflammation modulate asthmatic airway constriction?

An antigen challenge study

A. C. Henderson,¹ E. P. Ingenito,² H. Atileh,¹ E. Israel,² B. Suki,¹ and K. R. Lutchen¹

¹Department of Biomedical Engineering, Boston University, Boston 02215; and

²Pulmonary Division, Brigham and Women's Hospital, Boston, Massachusetts 02115

Submitted 27 January 2003; accepted in final form 16 April 2003

Henderson, A. C., E. P. Ingenito, H. Atileh, E. Israel, B. Suki, and K. R. Lutchen. Selected Contribution: How does airway inflammation modulate asthmatic airway constriction? An antigen challenge study. *J Appl Physiol* 95: 873–882, 2003. First published April 18, 2003; 10.1152/japplphysiol.00075.2003.—During the late-phase (LP) response to inhaled allergen, mediators from neutrophils and eosinophils are released within the airways, resembling what occurs during an asthma attack. We compared the distribution of obstruction and degree of reversibility that follows a deep inspiration (DI) during early-phase (EP) and LP responses in nine asthmatic subjects challenged with allergen. Heterogeneity of constriction was assayed by determining frequency dependence of dynamic lung resistance and elastance, airway caliber by tracking airway resistance during a DI, and airway inflammation by measuring inflammatory cells in induced sputum postchallenge. Despite a paucity of eosinophils in the sputum at baseline (<1% of nonsquamous cells), asthmatic subjects showed a substantial EP response with highly heterogeneous constriction and reduced capacity to maximally dilate airways. The LP was associated with substantial airway inflammation in all subjects. However, five subjects showed only mild LP constriction, whereas four showed more marked LP constriction characterized by heterogeneous constriction similar to EP. Bronchoconstriction during LP was fully alleviated by administration of a bronchodilator. These findings, together with the impaired bronchodilatory response during a DI, indicate a physiological abnormality in asthma at the smooth muscle level and indicate that airway inflammation in asthma is associated with a highly nonuniform pattern of constriction. These data support the hypothesis that variability in responsiveness among asthmatic subjects derives from intrinsic differences in smooth muscle response to inflammation.

allergen; airway reactivity

AIRWAY REACTIVITY IS A CARDINAL feature of asthma, but its precise relationship to inflammation and disease is poorly understood (27, 33, 34, 36). Although asthmatic subjects as a group display much greater constriction sensitivity to a nonspecific agonist compared with normal subjects, substantial overlap exists between nor-

mal and asthmatic subjects with regard to airway inflammation assayed by sputum cell counts, bronchoalveolar lavage, and airway biopsy sampling (20, 31, 34, 37). This suggests that the extent of airway inflammation may not dictate the phenotypic expression of asthma in a given patient and that phenotype, although related to inflammation, is principally dictated by other biological factors. This notion is supported by the recent findings of Crimi et al. (4), who showed that in patients with mild to moderate asthma, airway reactivity as measured by methacholine PC₂₀ [dose inducing 20% decline in 1-s forced expiratory volume (FEV₁)] correlated poorly with bronchoalveolar lavage eosinophil counts.

Alternatively, asthma phenotype may be dictated primarily at the level of the airway smooth muscle (ASM). To evaluate this, investigators have examined responses of asthmatic subjects to a deep inspiration (DI) to assay how ASM responds in vivo to stretch. Skloot et al. (29) demonstrated that, in healthy subjects, restricting DIs during a methacholine challenge makes airways become more reactive. However, among asthmatic subjects who are hyperreactive at baseline, inhibiting DIs did not have this effect (2). These findings support the concept that intrinsic differences in ASM physiology exist between normal and asthmatic subjects. Recently in humans, we applied a method to track airway resistance (Raw) as a real-time surrogate for airway caliber (17). We showed that asthmatic subjects are unable to maximally dilate their airways at baseline and that this physiological alteration worsens during induced constriction. These observations suggest an intrinsic defect in the contractile process at the ASM level. We also found that, in all cases, constriction was rapidly and completely reversed by administration of a β -agonist bronchodilator, suggesting that the β -receptor smooth muscle response in asthma is intact (17). How this apparent smooth muscle defect interacts with inflammation to produce the clinical features of asthma remains unknown.

Address for reprint requests and other correspondence: K. R. Lutchen, Dept. of Biomedical Engineering, Boston Univ., 44 Cummington St., Boston, MA 02215 (E-mail: klutch@bu.edu).

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.

An additional factor that is critically important in determining the severity of asthma is the distribution (i.e., heterogeneity and mean level) of constriction throughout the bronchial tree (22). Local, marked constriction affecting only a small percentage of airways can cause hypoxemia by altering ventilation-perfusion ($\dot{V}_A/\dot{Q}$) matching, can diminish dilator response to a DI by affecting local airway-parenchymal interdependence, and can produce refractoriness to inhaled medications by preventing their distribution to the most severely affected regions of lung. We have also recently shown that acute reactivity in asthmatic subjects creates this type of heterogeneous pattern of airway constriction (22) such that dynamic lung resistance and elastance (R_L and E_L , respectively) are highly elevated near breathing frequencies and are highly frequency dependent (19, 21, 25).

The goal of the present study was to evaluate the hypothesis that the asthmatic phenotype is determined principally by altered ASM function rather than by inherent differences in the degree of inflammation. If correct, then the pattern of constriction, dilatory response to a DI, and reversibility of constriction with bronchodilators should be similar during early-phase (EP) responses when minimal cellular inflammation is present and late-phase (LP) responses when substantial cellular inflammation is present. Conversely, if the extent of inflammation and airway wall edema dictates phenotypic variation, then physiological features of the constrictor response during EP and LP should be quite different.

METHODS

Subject selection. Nine asthmatic subjects were recruited for this study from the existing asthmatic patient population of the Asthma Research Center at Brigham and Women's Hospital. All subjects were adults (18–65 yr old), had no history of smoking or existing comorbid states (i.e., no immunocompromising condition, no cardiac disease by history, and not on anticoagulation therapy), and were not pregnant. Demographics for the subjects and their baseline FEV₁, methacholine PC₂₀, antigen used for challenge, and medications are shown in Table 1. Three subjects included in this

cohort did not undergo a methacholine challenge. Nevertheless, all three had experienced asthmatic symptoms and been diagnosed by a physician as having asthma. Subjects were asked to withhold their asthma medications as follows: short-acting β -agonists (e.g., albuterol) for 8 h; long-acting β -agonists (e.g., salmeterol) for 48 h; and Allegra, Claritin, or inhaled corticosteroids for 1 wk before the study. Subjects were told to consult with their physician about participating in the study and withholding their medications and were advised not to withhold their medications if they experienced any asthma symptoms. The Institutional Review Boards at Boston University and Brigham and Women's Hospital approved the study. Informed consent was obtained from each subject.

Experimental methods. We measured quasi-static pressure-volume curve data (QSPVC), R_L and E_L from 0.1 to 8 Hz, and Raw during tidal breathing and a DI to total lung capacity (TLC) in all subjects during EP and LP after antigen challenge and postalbuterol. To obtain R_L and E_L as a function of frequency, we used the optimal ventilator waveform (OVW) approach (23). The OVW is a broadband input flow waveform designed to mimic lung volumes during normal tidal breathing. The OVW flow signal is the sum of seven sinusoids combined to provide tidal-like ventilation while allowing for estimation of R_L and E_L between 0.15 and 8 Hz with the use of impedance analysis (18). Airway opening flow was measured with a pneumotachograph (Hans Rudolph series 4700A) connected to a pressure transducer (Celsco, model LCVR, 0–2 cmH₂O). Esophageal pressure was measured with a 10-cm latex balloon containing ~1 ml of air connected to a polyethylene catheter. Transpulmonary pressure (Ptp) was estimated as the difference between airway opening pressure and esophageal pressure and was measured across a single differential pressure transducer (Celsco, model LCVR, 0–50 cmH₂O). All signals were low-pass filtered at 10 Hz (4-pole Butterworth, Frequency Devices) and sampled at 40 Hz (Data Translations DT-2811 A/D board). An oxygen bias flow and soda lime scrubber device were used to prevent hypoxemia and hypercarbia during measurements.

Raw was tracked in real time by using the technique of Jensen et al. (17). A valve leading to a high-inertance tube was added to the breathing circuit and opened to the atmosphere to allow the subject to breathe spontaneously (17). The tube dimensions were chosen to function as a mechanical low-pass filter, allowing the subject to breathe fresh air

Table 1. Subject demographics

Subject No.	Gender	Age, yr	Height, in.	Weight, lb.	MCh PC ₂₀ , mg/ml	Antigen	Medication	Baseline FEV ₁ , %pred	EP FEV ₁ , %pred	LP FEV ₁ , %pred	Postalbuterol FEV ₁ , %pred
1*	M	36	71	185	0.254	Cat hair	Azmecort, albuterol	75	45	55	81
2	F	27	66.5	135	1.80	Dust mite	Albuterol	91	60	82	98
3	F	20	62.5	109	0.003	Dust mite	Flovent, Serevent Claritin, albuterol	92	70	83	107
4	M	22	64	175	2.01	Ragweed	Allegra, albuterol	109	74	82	116
5	F	30	62	132	3.57	Dust mite	Rhinocort, albuterol	113	66	94	114
6	M	23	72	185	NA	Tree mix	Albuterol	110	81	96	113
7	M	27	70	158	NA	Dust mite	Albuterol	103	57	99	109
8†	F	38	70	180	0.203	Cat hair	Albuterol	73	56	69	93
9	M	32	74	178	NA	Dust mite	Albuterol	98	79	63	88
Mean $\pm$ SE		28 $\pm$ 6	68 $\pm$ 4	160 $\pm$ 28	0.9 $\pm$ 1.3			96 $\pm$ 15	65 $\pm$ 12	80 $\pm$ 15	102 $\pm$ 13

Example subjects are classified by severity of late phase (LP): *severe, LP+, marked LP constriction; †mild, LP-, mild LP constriction. EP, early phase; N/A, data not available; M, male; F, female; MCh PC₂₀, methacholine dose causing 20% decline in 1-s forced expiratory volume (FEV₁); %pred, percent predicted.

spontaneously. The higher frequency 8-Hz oscillations generated by the piston pump were directed into the subject's lungs, permitting direct assessment of Raw by determining impedance. An on-line least squares algorithm was applied to a single-compartment model of the lung with RL and EL as time-varying parameters. The estimator was tuned so that RL reflected the resistance primarily from the most recent 8-Hz cycle and we assume $RL(8\text{ Hz}) = Raw(13, 15, 32)$.

Protocol. On the first day of the study, subjects were acclimated to the OVW system. Spirometry (FEV_1) was performed, and lung volumes were measured with a whole body plethysmograph (Morgan Scientific). Skin prick tests were used to determine antigen sensitivity. The antigen that produced the wheal (small, slightly reddened, circumscribed elevation of the skin) of greatest diameter and that was not in season was selected for subsequent inhalation challenge. Sputum induction was performed by using the technique of Fahy et al. (8) to assess the subject's baseline level of inflammation.

On the second day (separated by 2 days from *day 1*), a quantitative skin test was performed with the chosen antigen by using various dilutions (diluted with buffered saline) to determine the allergy sensitivity. A dose two serial dilutions below the most dilute dose that produced a wheal of at least 3 mm in diameter was used as the starting dose for the antigen challenge. An esophageal balloon was inserted, and baseline measurements were recorded. These included an OVW measurement, Raw tracking during tidal breathing and a DI, and a QSPVC. During the Raw tracking measurement, the patient was instructed to spontaneously breathe on the mouthpiece for four to five breaths, take a DI while keeping their glottis open, and then breathe for four to five more breaths. Subjects were instructed to inhale to TLC and then relax and exhale to residual volume on the mouthpiece while the technician periodically blocked the exhalation port for 2–3 s to generate a QSPVC.

Doses of the chosen antigen were prepared by using a serial dilution technique. First, 2 ml of buffered saline were injected into sterile rubber-capped vials. Next, 2 ml of the chosen allergen extract were injected into the first vial (1:2 dilution). After the solution was thoroughly mixed, 2 ml were removed and injected into the second vial (1:4 dilution). Serial dilutions were continued in this manner until the starting dose dilution (determined from quantitative skin test) was reached. Dosing of the antigen was then conducted until a 20% drop in FEV_1 below diluent baseline was observed, defined as the EP. An OVW measurement, Raw tracking, and a QSPVC were then performed.

After EP measurements, the esophageal balloon was removed. The subject's FEV_1 and transrespiratory Raw (i.e., respiratory system resistance at 8 Hz) were measured periodically (every 60 min) for the next 4–5.5 h to detect onset of any LP response. If the subject's FEV_1 dropped below 85% of the diluent baseline value, the subject was considered to have developed a LP response. Once this occurred, the esophageal balloon was reinserted, and OVW, Raw tracking, and QSPVC measurements were obtained. If the subject's FEV_1 did not drop below 85% of the diluent baseline value by 5.5 h after the initiation of the EP, measurements were made at that point. OVW and Raw tracking measurements were also repeated 4 min after the subject was given two puffs of albuterol at the end of the study to assess response to bronchodilator therapy. Finally, sputum induction was repeated to assess the level of inflammation during LP (8).

Data processing. The QSPVC data were fit by using the Salazaar and Knowles model (28). The model was fit to the

data by using a nonlinear least squares algorithm (3) that minimized the sum-of-squares of a given error function ($\sum e^2$). The error function was defined as $e = (Ptp^d - Ptp^m)$, where Ptp^d and Ptp^m are the transpulmonary pressure data and model, respectively. The static elastance (E_{stat}) was calculated as the inverse of the chord compliance between functional residual capacity and 500 ml above functional residual capacity. The lung impedance (ZL) and its coherence function (γ^2) were estimated by using the technique of Daroczy and Hantos (5). The dynamic RL was calculated from the real part (RC) as follows: $RL(f_k) = Re[ZL(f_k)]$, where k designates the frequency (f) of interest. The dynamic EL was calculated from the imaginary part (Im) as follows: $EL(f_k) = -2\pi f_k \cdot Im[ZL(f_k)]$.

The Ptp and flow measurements obtained during Raw tracking were digitally high-pass filtered (Butterworth, 4th order, cutoff frequency of 4 Hz) and applied to a one-compartment model using recursive least squares algorithm (forgetting factor = 0.92). The Raw tracking result was then plotted vs. time and against lung volume.

Data analysis. The E_{stat} , dynamic RL and EL, and Raw tracking measurements were compared at baseline, during EP and LP, and postalbuterol. The pattern of constriction was assessed by the frequency dependence of RL and EL, as performed by Lutchen et al. (22). From each Raw tracking measurement, the mean level of Raw before a DI (Raw^{pre-DI}) and the minimum Raw that a subject could achieve during an inspiration to TLC (Raw^{min}) were quantified. The Raw^{pre-DI} was used as a measure of the amount of airway constriction during tidal breathing, whereas Raw^{min} was used to assess a subject's maximum dilation capacity.

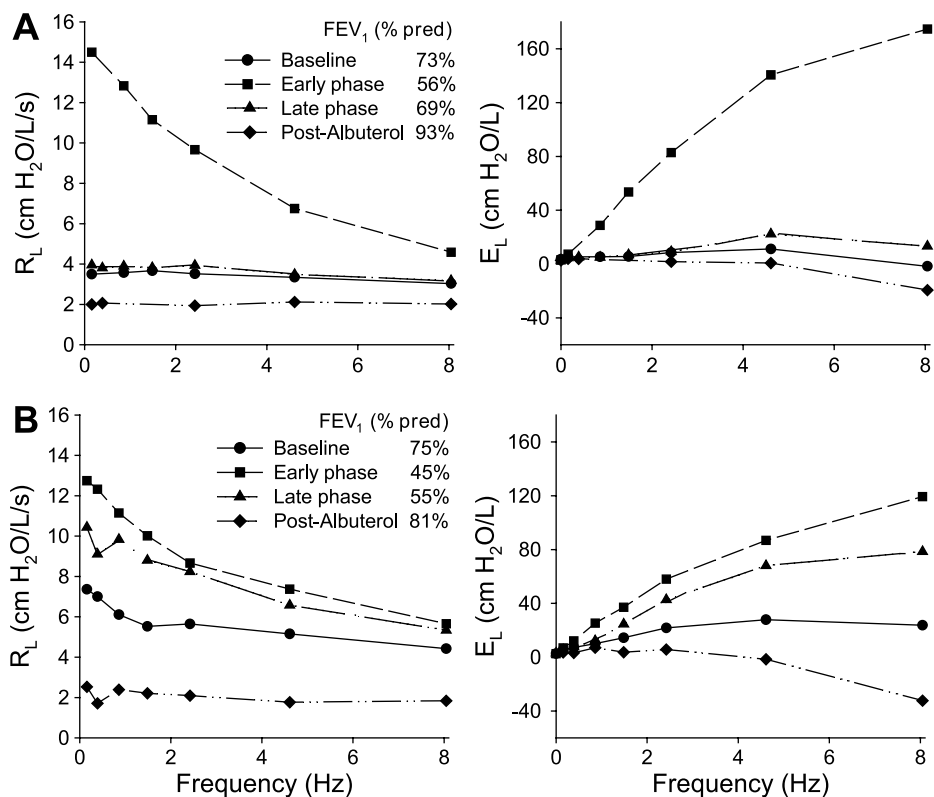
Sputum samples were processed according to the technique of Fahy et al. (8). Cells that were counted included squamous, macrophages, epithelial, neutrophils, eosinophils, and lymphocytes. At least 500 nonsquamous cells were counted. Samples having >80% squamous cells were not included because this indicates that the subject was producing cells from within the mouth and upper airways rather than from deep inside the lung. The percentage of the total nonsquamous cells was calculated for each type of nonsquamous cell. The level of inflammation was assessed for baseline and LP sputum slides as indicated by the percentage of neutrophils and eosinophils.

Statistical analysis. Comparisons between baseline, EP, LP, and postalbuterol measures of constriction heterogeneity, the bronchodilatory effect of a DI, and quasi-static lung properties were performed with a one-way ANOVA for repeated measures. If significance was obtained from ANOVA, a Tukey's post hoc analysis was performed. Within each group, parameter comparisons were performed with two-tailed paired *t*-tests. Statistical significance was defined as $P < 0.05$.

RESULTS

To illustrate the range of responses observed in this cohort, we first present the RL and EL data (Fig. 1) and sputum cell counts (Fig. 2) for two example asthmatic patients. One patient (Fig. 1A, *subject 8*) had mild obstruction at baseline (FEV_1 73% predicted), a pronounced EP response, and no LP response. The other patient (Fig. 1B, *subject 1*) had mild obstruction at baseline, (FEV_1 75% predicted), a substantial EP response, and an even more pronounced LP response. Similar to other asthmatic subjects, these example subjects showed evidence of a substantial and heterogeneous constriction response (Fig. 1) in EP. Interest-

Fig. 1. Constriction pattern as assessed by lung resistance (R_L) and lung elastance (E_L) vs. frequency for 2 example asthmatic subjects. *A*: subject 8 [mild late-phase (LP) constriction (LP-)]. *B*: subject 1 [marked LP constriction (LP+)]. FEV₁, forced expiratory volume in 1 s; %pred, percent predicted.



ingly, there was virtually no evidence of cellular inflammation in their sputum at baseline (eosinophils <1% of nonsquamous cells) (Fig. 2). At LP, both showed a significant and substantial increase in eosin-

ophils in sputum, but the mechanical responses relative to baseline ranged from none to severe. For example, Fig. 1A shows the R_L and E_L vs. frequency and Fig. 2A shows the cell count data for an asthmatic subject with a severe EP but no LP (LP-). At baseline, there were virtually no eosinophils. During the EP, this subject had substantial heterogeneous constriction, as indicated by a highly elevated level and frequency dependence of R_L and E_L . Nevertheless, at LP (6 h later), the R_L and E_L were minimally elevated from the baseline values. The R_L and E_L decreased below baseline values after administration of albuterol, presumably due to relaxation of airway tone below baseline. Cell counts showed that, compared with baseline, at LP the macrophages and lymphocytes decreased, whereas neutrophils and eosinophils increased.

Figure 1B shows example data from a subject with a positive LP (LP+). At baseline, this subject showed elevated R_L and E_L and elevated frequency dependence but few eosinophils in the sputum and only mild obstruction, as measured by spirometry. EP was accompanied by an increase in R_L and E_L at all frequencies and pronounced frequency dependence, suggesting marked heterogeneity of constriction. Five hours later, the eosinophil levels increased (Fig. 2B), and the subject had a LP manifest by marked constriction with a substantial increase in the R_L and E_L almost to the level of the EP response. After albuterol, R_L and E_L dropped to below baseline levels, indicating that the constriction resulting from LP response was immediately (within 3–5 min) relieved by smooth muscle relaxation.

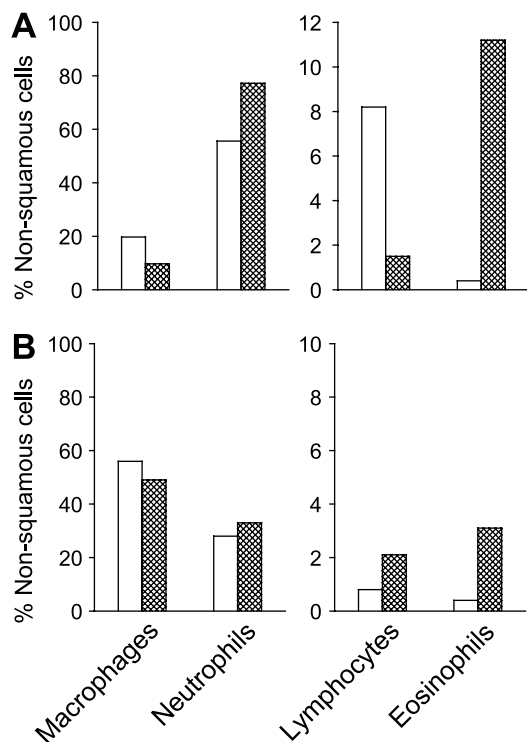


Fig. 2. Cell counts for 2 example asthmatic subjects. *A*: subject 8 (LP-). *B*: subject 1 (LP+).

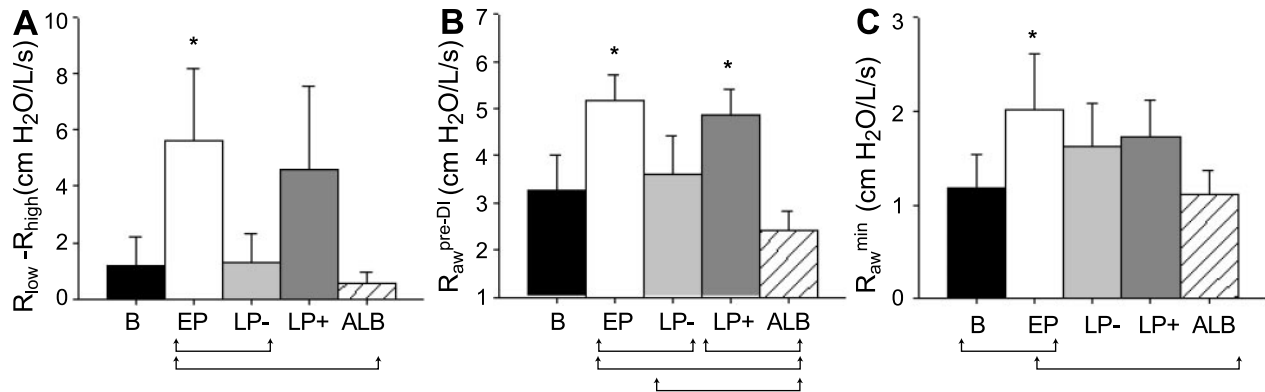


Fig. 3. Summary of RL and airway resistance (R_{aw}) tracking data for the asthmatic group ($n = 9$). A: heterogeneity. $R_{low} - R_{high}$, parameter reflecting overall frequency dependence of impedance. B: Raw. R_{aw}^{pre-DI} , mean level of Raw before a deep inspiration (DI). C: maximum airway caliber. R_{aw}^{min} , minimum Raw that a subject could achieve during an inspiration to total lung capacity. B, baseline; EP, early phase; Alb, albuterol. *Statistical significance relative to baseline, $P < 0.05$. Arrows show pairs that were statistically significant from Tukey's post hoc analysis. Note different scales.

Data for the entire study cohort are summarized with respect to magnitude and pattern changes of RL and EL, sputum cell counts, and airway dilation capacity data in response to a DI. Information about the extent of heterogeneity of constriction was expressed using the outcome parameter ($R_{low} - R_{high}$), which reflects overall frequency dependence of impedance. The capacity of ASM to dilate in response to mechanical stretch was evaluated with two indexes. First, the mean constriction level averaged over all airways was quantified via the mean Raw during tidal breathing (R_{aw}^{pre-DI}). Second, the maximum airway caliber a subject could achieve was quantified by the minimum Raw achievable (R_{aw}^{min}) following a DI.

Figure 3 summarizes these mechanical features in all subjects at baseline, EP, LP, and after albuterol on completion of LP measurements. Figure 4 summarizes the sputum assay of inflammation. For the LP, we distinguish between those subjects with a milder response ($FEV_1 > 85\%$ baseline after 5.5 h, LP-) and those with a more severe response ($FEV_1 < 85\%$ baseline after 5.5 h, LP+). During EP, all subjects had a rapid, substantial, and significant ($P < 0.05$) increase

in the heterogeneity of constriction ($R_{low} - R_{high}$), increase in R_{aw}^{pre-DI} , and decrease in the ability to maximally dilate airways (increase in R_{aw}^{min}). At baseline before administration of antigen and provocation of the EP response, the eosinophil counts were very low and neutrophil counts were in ranges previously reported for healthy subjects (1). These data indicate that there was little evidence of inflammation in the sputum across asthmatic subjects at baseline (just before EP). Thus airway hyperreactivity leading to a pronounced EP response occurred without inflammatory cells in the sputum.

At LP, every subject showed a substantial elevation in inflammatory cells in the sputum (neutrophils and eosinophils; $P < 0.05$) (Fig. 4). Nevertheless, not all subjects showed the same physiological phenotype. LP+ subjects developed marked and heterogeneous mechanical constriction during LP (Figs. 1 and 3) with a substantial increase in R_{aw}^{pre-DI} and evidence of a reduced capacity to maximally dilate airways in response to a deep breath (elevated R_{aw}^{min}). LP- subjects showed either no or mild constriction (R_{aw}^{pre-DI}) and no evidence of heterogeneity ($R_{low} - R_{high}$). However, as with the LP+ group, the LP- group also showed evidence of a reduction in capacity to maximally dilate airways (elevated R_{aw}^{min}). The elevated R_{aw}^{min} in both cases did not reach a level of statistical significance. Finally, even the severe LP responders, however, did not show a mechanical constriction response as substantial as what occurred at EP. In both LP+ and LP- subjects, albuterol administration brought lung mechanics back to baseline levels.

The averages and standard deviations of E_{stat} and maximum recoil pressure (P_{tp}^{max}) calculated from the QSPVCs are shown in Table 2. E_{stat} was not significantly different between baseline, EP, LP, and post-albuterol. P_{tp}^{max} was similar between baseline, LP, and post-albuterol time points. However, values were slightly lower immediately after antigen inhalation (EP) compared with at baseline ($P = 0.036$), suggesting

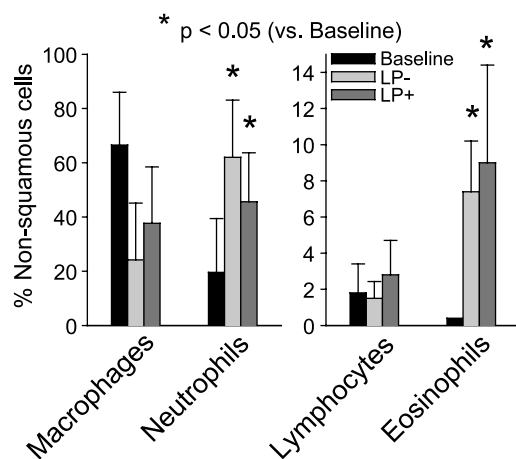


Fig. 4. Summary of cell count data for asthmatic group ($n = 9$).

Table 2. E_{stat} and Ptp^{max} for subjects at baseline, EP, LP, and postalbuterol

State	E_{stat} , cmH ₂ O/l	Ptp^{max} , cmH ₂ O
Baseline	3.3 ± 1.1	25 ± 7
Early phase	2.9 ± 1.3	$20 \pm 8^*$
LP+	2.9 ± 0.8	21 ± 3
LP-	4.0 ± 1.1	23 ± 7
Postalbuterol	3.1 ± 1.0	23 ± 6

E_{stat} , static elastance; Ptp^{max} , maximum recoil pressure. * $P < 0.05$, compared with baseline.

a shift in the QSPVC to higher volumes and subsequent small loss of recoil.

Tracking of the mechanical conditions between EP and LP was accomplished by measuring total respiratory resistance during tidal breathing ($R_{aw, tr}^{pre-DI}$) and at TLC ($R_{aw, tr}^{min}$) every 1 h. Figure 5 shows the response for the two example subjects highlighted in Figs. 1 and 2. At EP, both subjects had a large increase in $R_{aw, tr}^{pre-DI}$ and $R_{aw, tr}^{min}$, indicating constriction and loss of ability to maximally dilate airways during stretch. Between the EP and LP, these perturbations in lung mechanics returned to baseline levels. As LP developed, the subjects began to lose their ability to maximally dilate and $R_{aw, tr}^{pre-DI}$ became elevated. Note that constriction was not as severe as the EP, and albuterol brought the subjects down to baseline levels. It is interesting that the increases in $R_{aw, tr}^{pre-DI}$ and $R_{aw, tr}^{min}$ began ~3 h post-EP, whereas the abnormalities in spirometry were not evident until 6 h post-EP. Perhaps the increases in $R_{aw, tr}^{pre-DI}$ and $R_{aw, tr}^{min}$ are the initial and more sensitive indicators of the buildup in airway inflammation.

DISCUSSION

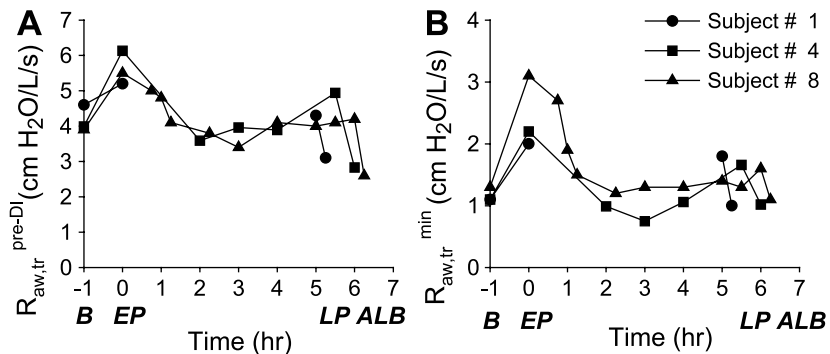
The primary objective of this study was to examine how the pattern and severity of airway constriction and the capacity to maximally dilate airways are related to airway inflammation in allergic asthma. Responses during both EP and LP were examined. LP responses were specifically targeted for study since the LP response is commonly considered to more accurately represent the spontaneous asthma phenotype in that it is a condition in which inflammation becomes elevated from the baseline EP. By combining measurements of static and dynamic lung mechanics, the

present study indicates that 1) bronchoconstriction postallergen challenge occurs independent of preexisting airway inflammation, as assayed by extent of sputum cellularity; 2) EP bronchoconstriction postallergen challenge is associated with markedly heterogeneous airway narrowing as assayed by frequency dependence of impedance (Figs. 1 and 3); 3) a positive LP reaction (as assessed via spirometry) also results in marked heterogeneity of constriction; 4) EP and LP postallergen challenges are associated with inability to fully dilate airways during stretch produced by maximal deep lung inflation and this is true whether the LP is + or - (Fig. 3); 5) Ptp generated during DI tend to be reduced during EP and LP; reductions in recoil pressure suggest that gas trapping may play a role in determining physiological responses in asthma; and 6) LP was uniformly associated with marked cellular inflammation and was not associated with the extent of bronchoconstriction, since both LP+ and LP- patients had similar increases in sputum eosinophils and neutrophils (Fig. 4).

Collectively, these observations have important implications with respect to disease severity in asthma and suggest a new paradigm for interpreting differences in phenotypic expression.

Haley and Drazen (12) have recently examined the relation between extent of active inflammation in the asthmatic airway and the tendency of that airway to bronchoconstrict after provocation. They proposed that there are two distinct ways through which inflammation can promote airway hyperreactivity (12). First, inflammatory cells (mast cells or perhaps standing inflammatory cells present in the submucosa but not in the airways) release histamine, leukotrienes, platelet-activating factor, and proteases that have short half-lives and can cause acute bronchoconstriction and increased airway responsiveness. Second, eosinophils can release major basic protein, which activates macrophages to release cytokines and chemokines that modify antigen processing and a response to β -agonists (12). Cytokines and chemokines remain in the airways for longer periods of time and may be responsible for airway remodeling and/or increased ASM tone. This hypothesis provides discrete immunologic pathways to explain asthmatic EP and LP responses and can explain why the extent of EP and LP responses in any given patient can be very different.

Fig. 5. Key features of transrespiratory R_{aw} ($R_{aw, tr}$) tracking data during an antigen challenge for 3 example asthmatic subjects. A: $R_{aw, tr}^{pre-DI}$. B: $R_{aw, tr}^{min}$.



Results of the present study are potentially consistent with this paradigm. EP responses were uniformly observed in all subjects, presumably in response to rapid release of histamine, leukotrienes, platelet-activating factor, and proteases from inflammatory cells in the submucosa that were not evident during assays of induced sputum samples at baseline. OVW measurements indicate that constriction during EP is extremely heterogeneous in its distribution. This is reflected in the marked frequency dependence of impedance and is consistent with our previous study showing that airway hyperreactivity in asthmatic patients creates a highly heterogeneous constriction phenotype in asthmatic patients (22). It is not clear whether this marked heterogeneity reflects a tendency for inflammatory cells to localize to specific sites within the lung or a tendency for airways to remodel in such a way that certain sites narrow to a greater extent than others when similarly challenged (10). Independent of mechanism, heterogeneity was universally observed during EP, and the ASM displayed hyperreactivity at baseline even without evidence of inflammation in the sputum.

The inability to maximally dilate airways during EP is thought to be a physiological characteristic of spontaneous asthma and could be due to several factors. First, in airways that are closed or significantly obstructed, airway-parenchymal interdependence may be lacking. In this case, the effects of a DI would be poorly transmitted to the airways within that lung zone. As a result, airways leading to those regions most significantly affected by airway narrowing would be least able to stretch during a deep inflation, resulting in overall attenuation of the DI bronchodilator response. Furthermore, the ability to maximally increase recoil pressure following constriction may be attenuated as a consequence of gas trapping resulting from the heterogeneous constriction pattern observed. The physiological consequences of heterogeneity may be even more pronounced in the clinical setting, since heterogeneity can cause hypoxemia as a result of $\dot{V}_A/\dot{Q}$ mismatching, providing a stimulus for tachypnea. As breathing rate increases, areas with long time constants progressively hyperinflate, and recoil pressure at TLC progressively declines, blunting the ability to maximally dilate during a DI. Finally, changes at the level of the ASM resulting from exposure to inflammatory mediators following antigen challenge may alter the intrinsic area transmural pressure relationship of ASM such that distensibility is attenuated.

There was no relationship observed between the severity of the EP and LP responses. In fact, some individuals with pronounced EP responses showed minimal LP responses with regard to heterogeneity and Raw^{pre-DI} but did show an elevated Raw^{min} (Fig. 3). Despite pronounced differences in extent of bronchoconstriction, however, all patients evaluated 5–6 h after antigen challenge displayed marked cellular inflammation in the airways assayed by induced sputum analysis. This observation suggests that physiological differences between individuals with asthma may not necessarily be due to differences in the extent of in-

flammation within the airways or to their ASM but rather to an individual's response to inflammation, a finding consistent with recent pharmacogenetic studies (6, 26).

Both EP and LP+ responses share two important characteristics, however. When a LP response was observed, it was characterized by marked heterogeneity and an inability to maximally dilate airways following a DI (although the latter did not reach statistical significance, the trend was clear). Specifically, the Raw^{pre-DI} and Raw^{min} in EP (5.2 and 2.0 $cmH_2O \cdot l^{-1} \cdot s$) were quite similar to the LP+ group (4.9 and 1.7 $cmH_2O \cdot l^{-1} \cdot s$, respectively). The reasons for this may be similar to those cited above in the discussion of EP responses. Independent of mechanism, these observations indicate that bronchoconstriction during both EP and LP is heterogeneous in nature, and this key physiological feature of the responses is independent of the extent of airway inflammation and may in part determine the inability to maximally dilate airways following a DI.

From sputum analysis, our asthmatic subjects displayed virtually no evidence of intraluminal eosinophils at baseline (EP) and a substantial elevation at LP. Unfortunately, sputum analysis cannot assay inflammation within the airway wall. During LP, other inflammatory conditions likely exist not explicitly evident from sputum results. For example, one might expect airway swelling and/or thickening that could enhance peripheral resistance. Also, inflammatory cells are known to stimulate a mixture of endogenous mediators that are bronchoconstrictive (12). These mediators are distinct from the mast cell histamine response that occurs during EP. Although we do not know the explicit inflammatory events occurring *in vivo*, we do know that the sum total of inflammatory conditions at LP always reduced the ability to maximally dilate airways (i.e., elevated the R_{min}) and occasionally created heterogeneous constriction conditions akin to EP. Nevertheless, there was always a full recovery of R_{min} and any elevated frequency dependence (i.e., heterogeneity) after bronchodilator-induced relaxation of smooth muscle. Hence, amplified constriction conditions at LP appear to derive from inflammation-induced abnormal constriction in the ASM, rather than from swelling or thickening *per se*.

The diversity of LP responses among patients with seeming equivalent degrees of EP response and clinical asthma is also of interest and may be due to several factors. It seems likely that, given the time course of the EP response in relation to antigen exposure, mast cell activation likely played an important role. Mediators such as histamine, tryptase, and prostanoids released from mast cell granules following IgE activation would thus modulate the physiological response of the EP. The lack of detectable inflammatory cells in induced sputum is consistent with this notion, since these cells remain submucosal and are difficult to detect by this assay. By contrast, inflammatory mediators released from eosinophils and neutrophils may modulate LP responses, and the tendency to broncho-

constrict in response to this panel of mediators may be somewhat more variable. Nevertheless, in both cases, the inability to dilate in response to a deep breath points to a defect at the level of the smooth muscle that may be due to changes resulting from long-term and/or repetitive exposure to inflammatory mediators. Several groups have reported that reductions in periodic lengthening of smooth muscle for extended periods of time will likely cause remodeling of the muscle contractile apparatus, resulting in a muscle state that is stiffer and more contractile (9, 11, 35). If correct, this would suggest that inflammation is capable of altering ASM phenotype via chronic exposure to a milieu of endogenous mediators that sustain increased muscle tone. However, our results indicate that such an alteration does not affect the ability of smooth muscle to undergo rapid relaxation following β -agonist administration.

Most investigators would concur that increased airway inflammation will generally exacerbate the severity of asthma. The results presented here suggest, however, that the relationship between inflammation and asthma severity is not straightforward. To date, few studies have provided an explicit scenario for how chronic inflammation might impact function or, more specifically, severity and hyperreactivity in asthmatic patients. It is generally accepted that asthmatic subjects constrict more than healthy subjects when exposed to a given (nonspecific) agonist and respond at a lower dose (2). The mechanisms that other studies have proposed recently to explain why asthmatic subjects are hyperreactive have focused primarily on ASM biology and include the following: 1) the ASM is in a stiffer and stronger state (9), 2) the load conditions allow the ASM to maximally shorten (reduced tethering) (24), 3) inflammatory mediator release promoting airway reactivity is more pronounced (16), 4) airway edema (7, 40), mucus plugging (7), and airway wall thickening (inflammation and/or remodeling) amplify the amount of luminal reduction for a given amount of ASM shortening (38), 5) surfactant dysfunction (14, 39) leads to narrowing and collapse of small airways, and 6) the epithelial cells are more transparent in asthma, allowing more agonist to reach the ASM (30). Our results do not directly support or dispel any of these mechanisms but suggest that, independent of the biological pathway involved, heterogeneity is a key feature of the response and heterogeneity itself may determine key phenotypic characteristics of asthma. It is worth noting that the increase in frequency dependence in R_L simultaneously with the large increase in airway wall shunting (increase in E_L at 8 Hz) points to the periphery of the lung as the major site of constriction. This constriction likely derives from a combination of excessive ASM shortening simultaneously with amplified airway wall swelling or thickening.

Limited ability to maximally dilate airways after a DI may in part relate to the reduction in Ptp that results from gas trapping and an inability to fully transmit Ptp changes to areas that are poorly ventilated. It is also possible that, in extreme cases, heterogeneity can explain refractoriness to medical therapy.

Areas involving airways that are maximally constricted are also poorly ventilated; therefore, these areas will receive little inhaled medication. This may be further exacerbated by dilation in less severely obstructed areas, since this could result in further $\dot{V}_A/\dot{Q}$ mismatching, worsening hypoxemia, and a greater stimulus for tachypnea and gas trapping.

Could it be argued that the ASM is similar in healthy and asthmatic subjects but that asthmatic hyperreactivity is simply a consequence of more bronchoconstrictive mediators getting to the smooth muscle? Perhaps, but it is well known that nonsymptomatic asthmatic subjects without baseline evidence of enhanced inflammation are hyperreactive to nonspecific constrictive agonists. Also, in our specific study, a severe constrictive EP response occurred with minimal evidence of inflammation in the airway lumen. Thus airways are hyperreactive (to the acute, histamine-like stimulus at EP), and excessive inflammation was not a prerequisite. A question then arises as to why, with similar degrees of enhanced inflammation, some subjects are LP+, whereas others are LP-. Here, the ASM in LP- asthmatic subjects does not have to be inherently different from the ASM in LP+ asthmatic subjects. Rather, at LP, the mediator systems are complex and, in some subjects, sufficient to induce a hyperreactive ASM response (akin to that seen at EP), whereas in others it is not. In the end, it appears that the ASM must be hyperreactive before the onset of "chronic-like" inflammation and in a manner not present in healthy subjects.

One additional feature of the present study deserves comment. It is remarkable that, even in those instances where a pronounced LP was observed, bronchoconstriction was rapidly and completely reversed with albuterol. These findings suggest that airway inflammation resulting from antigen inhalation exerts its physiological effect by altering ASM tone rather than by causing mucus plugging, thickening of the airway wall by producing edema, or alteration of airway surfactant through protein leakage. This conclusion is supported by the fact that β -agonist therapy not only reversed bronchoconstriction but also, in virtually all instances, lowered R_{aw} to below preallergen baseline levels. This suggests that baseline tone in the asthmatic airway is increased and that both EP and LP constriction in this study specifically reflects the physiological response of the ASM.

The primary goal of this study was to probe how airway reactivity in asthmatic subjects during acute stimulation (EP) compared with airway constriction conditions that developed as a consequence of inflammatory cells trafficking to the airway system (LP). This required that we use atopic asthmatic subjects that served as their own controls. It is not evident whether data from atopic, but nonasthmatic normal subjects would aid in interpretation of these data. By definition, atopic normal subjects would show a heterogeneous early response to some antigen. However, because such subjects would not be asthmatic, they would not (by definition) have airway hyperreactivity to a nonspecific

agonist. Hence, if a normal subject showed no enhancement in inflammatory cells (i.e., no LP), then one can say nothing regarding the role of inflammation in asthma. Even if atopic normal subjects did show enhanced inflammation at LP, all we could evaluate would be how such inflammation impacts their mechanical conditions and not those of asthmatic patients per se. Our concern was the impact of enhanced inflammation in the lung of an individual that already has asthma and has airway hyperreactivity even to non-specific stimuli.

In summary, the data presented here suggest that allergen exposure causes EP and LP constriction primarily by altering ASM tone. The data further suggest that there is a poor relationship between the extent of inflammation in the airways assayed by induced sputum and the extent of bronchoconstriction. Finally, the results suggest that heterogeneity and reduced capacity to maximally dilate airways are key features of the physiological response. In fact, these features may in part determine the diminished responsiveness to inhaled medical therapy in some cohorts of severe asthmatic patients (37). Whether physiological heterogeneity is due to the presence of focal sites of inflammation within the airways, differences in ASM density at specific sites within the airways, or local narrowing resulting from airway remodeling is not clear, but defining the biological basis for this pronounced and consistent heterogeneity and the diminished airway dilatory response may provide insight into key features of the asthmatic phenotype, thus being worthy of further investigation.

DISCLOSURES

This work was supported by National Heart, Lung, and Blood Institute Grant HL-62269-02 and the National Science Foundation, Bioengineering Division.

REFERENCES

- Belda J, Leigh R, Parameswaran K, O'Byrne PM, Sears MR, and Hargreave FE. Induced sputum cell counts in healthy adults. *Am J Respir Crit Care Med* 161: 475–478, 2000.
- Brusasco V, Crimi E, Barisione G, Spanevello A, Rodarte JR, and Pellegrino R. Airway responsiveness to methacholine: effects of deep inhalations and airway inflammation. *J Appl Physiol* 87: 567–573, 1999.
- Coleman TF and Li Y. On the convergence of reflective Newton methods for large-scale nonlinear minimization subject to bounds. *Mathematical Programming* 67: 189–224, 1994.
- Crimi E, Spanevello A, Neri M, Ind PW, Rossi GA, and Brusasco V. Dissociation between airway inflammation and airway hyperresponsiveness in allergic asthma. *Am J Respir Crit Care Med* 157: 4–9, 1998.
- Daroczy B and Hantos Z. An improved forced oscillatory estimation of respiratory impedance. *Int J Biomed Comput* 13: 221–235, 1982.
- Drazen J. Clinical pharmacology of leukotriene receptor antagonists and 5-lipoxygenase inhibitors. *Am J Respir Crit Care Med* 157: S233–S237, 1998.
- Fahy J, Corry DB, and Boushey HA. Airway inflammation and remodeling in asthma. *Curr Opin Pulm Med* 6: 15–20, 2000.
- Fahy J, Boushey HA, Lazarus SC, Mauger EA, Cherniack RM, Chinchilli VM, Craig TJ, Drazen JM, Ford JG, Fish JE, Israel E, Kraft M, Lemanske RF, Martin RJ, McLean D, Peters SP, Sorkness C, and Szeffler SJ. Safety and reproducibility of sputum induction in asthmatic subjects in a multicenter study. *Am J Respir Crit Care Med* 163: 1470–1475, 2001.
- Fredberg J, Jones KA, Raboudi NS, Prakash YS, Shore SA, Butler JP, and Sieck GC. Friction in airway smooth muscle: mechanism, latch, and implications in asthma. *J Appl Physiol* 81: 2703–2712, 1996.
- Gillis H and Lutchen KR. Airway remodeling in asthma amplifies heterogeneities in smooth muscle shortening causing hyperresponsiveness. *J Appl Physiol* 86: 2001–2012, 1999.
- Gunst S and Tang DD. The contractile apparatus and mechanical properties of airway smooth muscle. *Eur Respir J* 15: 600–616, 2000.
- Haley K and Drazen JM. Inflammation and airway function in asthma: what you see is not necessarily what you get. *Am J Respir Crit Care Med* 157: 1–3, 1998.
- Hantos Z, Daroczy B, Suki B, Nagy S, and Fredberg JJ. Input impedance and peripheral inhomogeneity of dog lungs. *J Appl Physiol* 72: 168–178, 1992.
- Hohlfeld J, Fabel H, and Hamm H. The role of pulmonary surfactant in obstructive airways disease. *Eur Respir J* 10: 482–491, 1997.
- Ingenito E, Davison B, and Fredberg JJ. Tissue resistance in the guinea pig at baseline and during methacholine constriction. *J Appl Physiol* 75: 2541–2548, 1993.
- Jarjour N, Calhoun WJ, Kelly EAB, Gleich GJ, Schwartz LB, and Busse WW. The immediate and late allergic response to segmental bronchopulmonary provocation in asthma. *Am J Respir Crit Care Med* 155: 1515–1521, 1997.
- Jensen A, Atileh H, Suki B, Ingenito EP, and Lutchen KR. Airway caliber in healthy and asthmatic subjects: effects of bronchial challenge and deep inspirations. *J Appl Physiol* 91: 506–515, 2001.
- Kaczka D, Ingenito EP, Suki B, and Lutchen KR. Partitioning airway and lung tissue resistances in humans: effects of bronchoconstriction. *J Appl Physiol* 82: 1531–1541, 1997.
- Kaczka D, Ingenito EP, Israel E, and Lutchen KR. Airway and lung tissue mechanics in asthma: effects of albuterol. *Am J Respir Crit Care Med* 159: 169–178, 1999.
- Louis R, Lau LCK, Bron AO, Roldaan AC, Radermecker M, and Djukanovic R. The relationship between airways inflammation and asthma severity. *Am J Respir Crit Care Med* 161: 9–16, 2000.
- Lutchen K and Gillis H. Relationship between heterogeneous changes in airway morphometry and lung resistance and elastance. *J Appl Physiol* 83: 1192–1201, 1997.
- Lutchen K, Jensen A, Atileh H, Kaczka DW, Israel E, Suki B, and Ingenito EP. Airway constriction pattern is a central component of asthma severity: the role of deep inspirations. *Am J Respir Crit Care Med* 164: 207–215, 2001.
- Lutchen K, Yang K, Kaczka DW, and Suki B. Optimal ventilator waveforms for estimating low-frequency respiratory impedance. *J Appl Physiol* 75: 478–488, 1993.
- Macklem P. Mechanical factors determining maximum bronchoconstriction. *Eur Respir J Suppl* 6: 516s–519s, 1989.
- Otis A, McKerrow CB, Bartlett RA, Mead J, McIlroy MB, Selverstone NJ, and Radford EP Jr. Mechanical factors in distribution of pulmonary ventilation. *J Appl Physiol* 8: 427–443, 1956.
- Palmer L, Silverman ES, Weiss ST, and Drazen JM. Pharmacogenetics of asthma. *Am J Respir Crit Care Med* 165: 861–866, 2002.
- Pliss L, Ingenito EP, and Ingram RH Jr. Responsiveness, inflammation, and effects of deep breaths on obstruction in mild asthma. *J Appl Physiol* 66: 2298–2304, 1989.
- Salazar E and Knowles JH. An analysis of pressure-volume characteristics of the lungs. *J Appl Physiol* 19: 97–104, 1964.
- Skloot G, Permutt S, and Toghias A. Airway hyperresponsiveness in asthma: a problem of limited smooth muscle relaxation with inspiration. *J Clin Invest* 96: 2393–2403, 1995.
- Sparrow M, Omari TI, and Mitchell HW. The epithelial barrier and airway responsiveness. *Can J Physiol Pharmacol* 73: 180–190, 1995.
- Ten Brinke A, Zwinderman AH, Sterk PJ, Rabe KF, and Bel EH. Factors associated with persistent airflow limitation

- in severe asthma. *Am J Respir Crit Care Med* 164: 744–748, 2001.
32. **Tepper R, Sato J, Suki B, Martin G, and Bates JHT.** Low-frequency pulmonary impedance in rabbits and its response to inhaled methacholine. *J Appl Physiol* 73: 290–295, 1992.
 33. **Van Den Berge M, Kerstjens HAM, Meijer RJ, De Reus DM, Koeter GH, Kauffman HF, and Postma DS.** Corticosteroid-induced improvement in the PC₂₀ of adenosine monophosphate is more closely associated with reduction in airway inflammation than improvement in the PC₂₀ of methacholine. *Am J Respir Crit Care Med* 164: 1127–1132, 2001.
 34. **Van Den Toorn L, Overbeek SE, De Jongste JC, Leman K, Hoogsteden HC, and Prins J.** Airway inflammation is present during clinical remission of atopic asthma. *Am J Respir Crit Care Med* 164: 2107–2113, 2001.
 35. **Wang L, Pare PD, and Seow CY.** Changes in force-velocity properties of trachealis due to oscillatory strains. *J Appl Physiol* 92: 1865–1872, 2002.
 36. **Ward C, Johns DP, Bish R, Pais M, Reid DW, Ingram C, Feltis B, and Walters EH.** Reduced airway distensibility, fixed airflow limitation, and airway wall remodeling in asthma. *Am J Respir Crit Care Med* 164: 1718–1721, 2001.
 37. **Wenzel S, Schwartz LB, Langmack EL, Halliday JL, Trudeau JB, Gibbs RL, and Chu HW.** Evidence that severe asthma can be divided pathologically into two inflammatory subtypes with distinct physiologic and clinical characteristics. *Am J Respir Crit Care Med* 160: 1001–1008, 1999.
 38. **Wiggs B, Moreno R, Hogg JC, Hilliam C, and Pare PD.** A model of the mechanics of airway narrowing. *J Appl Physiol* 69: 849–860, 1990.
 39. **Wright S, Hockey PM, Enhorning G, Strong P, Reid KBM, Holgate ST, Djukanovic R, and Postle AD.** Altered airway surfactant phospholipid composition and reduced lung function in asthma. *J Appl Physiol* 89: 1283–1292, 2000.
 40. **Yager D, Kamm RD, and Drazen JM.** Airway wall liquid. *Chest* 107: 105S–110S, 1995.

