Airway Hyperresponsiveness: From Molecules to Bedside

Selected Contribution: Small airway morphology and lung function in the transition from normality to chronic airway obstruction

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Corsico, Angelo, Manlio Milanese, Simonetta Baraldo, Gian Luca Casoni, Alberto Papi, Anna Maria Riccio, Isa Cerveri, Marina Saetta, and Vito Brusasco. Selected Contribution: Small airway morphology and lung function in the transition from normality to chronic airway obstruction. J Appl Physiol 95: 441–447, 2003. First published February 21, 2003; 10.1152/japplphysiol.01018.2002.—This study investigated the relationships between pathological changes in small airways (<6 mm perimeter) and lung function in 22 nonasthmatic subjects (20 smokers) undergoing lung resection for peripheral lesions. Preoperative pulmonary function tests revealed airway obstruction [ratio of forced expiratory volume in 1 s to forced vital capacity (FEV1/FVC) < 70%] in 12 subjects and normal lung function in 10. When all subjects were considered together, total airway wall thickness was significantly correlated with FEV1/FVC (r2 = 0.25), reactivity to methacholine (r2 = 0.26), and slope of linear regression of FVC against FEV1 values recorded during the methacholine challenge (r2 = 0.56). Loss of peribronchialolar alveolar attachments was significantly associated (r2 = 0.25) with a bronchoconstrictor effect of deep inhalation, as assessed from a maximal-to-partial expiratory flow ratio <1, but not with airway responses to methacholine. No significant correlation was found between airway smooth muscle thickness and lung function measurements. In conclusion, this study suggests that thickening of the airway wall is a major mechanism for airway closure, whereas loss of airway-to-lung interdependence may contribute to the bronchoconstrictor effect of deep inhalation in the transition from normal lung function to airway obstruction in nonasthmatic smokers.

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from lobar resections on patients with lung function ranging from normality to overt chronic airway obstruction were analyzed. The morphometric data were compared with the effects of deep inhalation and airway responses to methacholine (MCh) determined preoperatively.

**METHODS**

**Subjects**

The study included 22 subjects (Table 1) undergoing thoracotomy and lobar resection for peripheral pulmonary nodule. Twelve of them were current smokers, eight were past smokers, and two never smoked. Thirteen subjects (12 smokers and 1 past smoker) had a history of chronic bronchitis, and none had a history of bronchial asthma. To be included, subjects were required not to show any radiographic sign of invasion of large airways or pleura by the tumor, potentially influencing overall lung mechanics. At the time of the study, all subjects were in stable clinical conditions, and none of them was taking regular treatment with steroids or bronchodilator agents. Short-acting \( \beta_2 \)-agonists on demand, if any, were avoided for 12 h before pulmonary function tests. The study was approved by the local ethics committees, and informed consent was obtained from each subject.

**Lung Function Measurements**

A Vmax 22 system (SensorMedics, Yorba Linda, CA) or a Baires system (Biomedin, Padua, Italy) was used for lung function measurements. FEV1 and FVC were determined according to the American Thoracic Society criteria (2) and compared with the predicted values of Quanjer et al. (33). Measurements of single-breath diffusion capacity for carbon monoxide were obtained in 19 subjects and compared with the predicted values from Cotes et al. (7).

**Effect of deep inhalation at control**

Partial expiration (PEFV) and maximal expiration (MEFV) flow-volume curves were obtained after at least 2 min of regular tidal breathing by asking the subjects to expire forcefully from \( \sim 70\% \) FVC to residual volume (RV) (PEFV maneuver), to inspire immediately and fast to total lung capacity (TLC), and, without breath holding, to expire again forcefully to RV (MEFV maneuver). Expiratory flows were measured at 40\% FVC on both MEFV and PEFV, and their ratio (M/P\text{ov}) was taken as an index of the effect of deep inhalation on airway caliber. In this analysis, M/P\text{ov} values of 1 indicate no effect of deep inhalation on airway caliber, whereas values \( >1 \) and \( <1 \) indicate bronchodilator and bronchoconstrictor effects, respectively.

**Airway responsiveness and air trapping**

A MCh inhalation challenge was used to induce bronchoconstriction in 15 consenting subjects who met the safety criteria for MCh bronchial challenge (1). Dry powder MCh (Laboratorio Farmaceutico Lofarma, Milan, Italy) was dissolved in distilled water and aerosolized by a breath-activated dosimeter system (Mefar, Brescia, Italy) driven by compressed air and set to deliver 10 \( \mu L \) of solution per actuation. Subjects were asked to maintain their spontaneous tidal breathing pattern and refrain from taking deep breaths during MCh inhalation. The starting dose of MCh was of 50 \( \mu g \), and double increments were obtained by varying the number of inhalations, the MCh concentration, or both. The test ended when the FEV1 decreased by \( \geq 20\% \) of control or a noncumulative dose of 3,200 \( \mu g \) had been achieved. At the end of challenge, subjects were given salbutamol (200 \( \mu g \), by metered dose inhaler) and left the laboratory after their FEV1 had returned to within 10\% of their control value.

Airway responsiveness was inferred from the slope of the MCh dose-response curve, i.e., the FEV1 percent decrements plotted against MCh log doses, to obtain a continuous variable available in all subjects. Because no plateau was observed in any dose-response curves, all the data points available (4 to 8) for each subject were included in the analysis. For those subjects who achieved an FEV1 decrease \( \geq 20\% \), the provocative dose causing a 20\% FEV1 decrease (PD\text{av}) was also calculated by linear interpolation between two adjacent points of the dose (log)-response curve.

The occurrence of air trapping was inferred by regressing all values of FVC against the corresponding values of FEV1 recorded during the MCh challenge (6). Particular attention was paid to the achievement of end-of-test criteria for FVC (2) at each step of the challenge. In this analysis, a slope of zero indicates that MCh decreases FEV1 without affecting FVC, thus suggesting occurrence of airway narrowing without air trapping. By converse, slope values \( >1 \) and high intercepts indicate more air trapping than airway narrowing.

**Histology**

Four to six randomly selected tissue blocks (template size \( 1 \times 2 \times 2 \mathrm{~cm} \)) were taken from the subpleural parenchyma of the lobe obtained from surgery, distant from the area of the pulmonary nodule. Samples were fixed in 4\% formaldehyde in phosphate-buffered saline at pH 7.2 and, after dehydration, embedded in paraffin wax. Tissue specimens were properly oriented, and 5-\( \mu \mathrm{m} \)-thick serial sections were cut for morphometric analysis.

Sections were stained with hematoxylin-eosin and analyzed by light microscopy (Leica DMLB, Leica, Cambridge, UK) connected to a video recorder and a computerized image system (Casti Imaging SC processing software), as previously described (35). Briefly, for each subject all noncartilagineous peripheral airways with an internal perimeter <6 mm (corresponding to an airway diameter of <2 mm) were examined, and their morphological data were averaged. Bronchioles with a short-to-long diameter ratio of less than one-third were excluded to avoid measurements in tangentially cut airways. The internal perimeter along the subepithelial basement membrane (\( \mathrm{P}_{\text{bas}} \)) and the luminal diameter in a plane perpendicular to the long axis of the lumen (\( \mathrm{D}_l \)) were measured. In the presence of mucosal folds, \( \mathrm{D}_l \) was measured as the distance between the crest of one fold and the valley of
the facing fold on the opposite side. Total wall area (WA<sub>tot</sub>), i.e., everything between basement membrane and external wall border, and smooth muscle area (WA<sub>sm</sub>) were measured. Values of D<sub>h</sub>, WA<sub>tot</sub>, and WA<sub>sm</sub> were normalized for P<sub>bm</sub>, which is considered to be a reliable marker of airway size that remains constant with changes in smooth muscle tone and lung volume (17).

Alveolar attachments (AA), i.e., the alveolar septa that extend radially from the outer wall of nonrespiratory bronchioles (36), were counted over the external circumference. Any AA showing discontinuity from the peribronchial layer or rupture was denoted as a destroyed attachment (AA<sub>d</sub>, Fig. 1). The numbers of intact AA (AA<sub>i</sub>) per millimeter of airway external perimeter and the percentage of AA<sub>d</sub> to total AA were calculated.

Cases were coded and measurements were made by the same reader, who was unaware of clinical data.

**Statistical Analysis**

Data are presented as means ± SD, unless otherwise indicated. Relationships between variables were tested by Pearson’s correlation. When a functional variable correlated with more than one morphological variable by univariate analysis, their simultaneous effects were analyzed by multiple linear regression analysis with independent variables selected by a stepwise procedure. Student’s t-test was also used when appropriate. Values of P ≤ 0.05 were considered statistically significant.

**RESULTS**

Of the 22 subjects who took part in the study, 12 had airflow obstruction (FEV<sub>1</sub>/FVC <70%). In six obstructed subjects, there was a moderate to severe reduction of single-breath diffusion capacity for carbon monoxide (between 47 and 66% of predicted). The thickness of total airway wall area (WA<sub>tot</sub>/P<sub>bm</sub>) was significantly greater in subjects with than in those without airflow obstruction (Table 2) and negatively correlated (r = -0.50, P < 0.05) with the FEV<sub>1</sub>-to-FVC ratio when data were pooled.

**Effect of Deep Inhalation at Control**

The M/P<sub>40</sub> was <1 in 18 of the 22 subjects, with no significant difference between those with (0.73 ± 0.28) and those without (0.87 ± 0.24) airflow obstruction (P = 0.23). When all subjects were considered together, M/P<sub>40</sub> was found to be negatively correlated (r = -0.48, P < 0.05) with the percentage of AA<sub>d</sub> (Fig. 2A) and positively correlated (r = 0.50, P < 0.05) with the number of AA<sub>i</sub> (Fig. 2B), although the significance of the latter correlation was apparently due to one outlying subject.

**Airway Responsiveness and Air Trapping**

The slope of FVC vs. FEV<sub>1</sub> was barely correlated (r = 0.22, P = 0.48) or AA<sub>i</sub> (r = -0.18, P = 0.55). In eight subjects in whom FEV<sub>1</sub> decreased by 20% or more, PD<sub>20</sub> was significantly correlated (r = 0.87, P < 0.05) with D<sub>h</sub>/P<sub>bm</sub> (Fig. 4) but not with other morphometric data.

The slope of FVC vs. FEV<sub>1</sub> was significantly steeper (1.72 ± 0.22 vs. 1.10 ± 0.26, P < 0.001) and the intercept tended to be lower (−0.25 ± 0.47 vs. 0.47 ± 0.71, P = 0.05) in subjects with (n = 8) than in those without (n = 6) airflow obstruction, indicating more air trapping in the former. One obstructed subject was not able to meet the end-of-test criteria on most steps of the challenge and was excluded from this analysis. When all subjects were considered together, the slope of FVC vs. FEV<sub>1</sub> was found to be positively correlated (Fig. 5) with both WA<sub>tot</sub>/P<sub>bm</sub> (r = 0.75, P < 0.005) and WA<sub>sm</sub>/P<sub>bm</sub> (r = 0.74, P < 0.005), but only WA<sub>tot</sub>/P<sub>bm</sub> was retained by stepwise multiple regression analysis. No

**Table 2. Airway morphology**

<table>
<thead>
<tr>
<th></th>
<th>Obstructed (n = 12)</th>
<th>Nonobstructed (n = 10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>P&lt;sub&gt;bm&lt;/sub&gt;, mm</td>
<td>1.25 ± 0.40</td>
<td>1.62 ± 0.72</td>
</tr>
<tr>
<td>D&lt;sub&gt;h&lt;/sub&gt;/P&lt;sub&gt;bm&lt;/sub&gt;</td>
<td>0.16 ± 0.05</td>
<td>0.13 ± 0.04</td>
</tr>
<tr>
<td>WA&lt;sub&gt;tot&lt;/sub&gt;/P&lt;sub&gt;bm&lt;/sub&gt;, μm</td>
<td>85 ± 27</td>
<td>61 ± 24*</td>
</tr>
<tr>
<td>WA&lt;sub&gt;sm&lt;/sub&gt;/P&lt;sub&gt;bm&lt;/sub&gt;, μm</td>
<td>13 ± 12</td>
<td>7 ± 2</td>
</tr>
<tr>
<td>AA&lt;sub&gt;i&lt;/sub&gt;, %</td>
<td>46 ± 20</td>
<td>46 ± 20</td>
</tr>
<tr>
<td>AA/P&lt;sub&gt;n&lt;/sub&gt;, n/mm</td>
<td>6.0 ± 4.3</td>
<td>4.7 ± 2.6</td>
</tr>
</tbody>
</table>

Values are means ± SD. P<sub>bm</sub>, perimeter of basement membrane; D<sub>h</sub>, luminal diameter; WA<sub>tot</sub>, total wall area; WA<sub>sm</sub>, smooth muscle area; AA<sub>d</sub>, destroyed alveolar attachments; AA<sub>i</sub>, intact alveolar attachments; P<sub>n</sub>, airway external perimeter. *P < 0.05 vs obstructed.
significant correlation was found between air trapping and $\text{AA}_d \ (r = 0.44, \ p = 0.15)$ or $\text{AA}_i \ (r = -0.17, \ p = 0.59)$.

**DISCUSSION**

The major findings of this study in a group of subjects with lung function ranging from normality to overt airway obstruction are the following: 1) the loss of alveolar attachments to airway walls was significantly associated with a bronchoconstrictor effect of deep inhalation but not with airway hyperreactivity or air trapping, 2) thickening of total airway wall was associated weakly with airway hyperreactivity and strongly with air trapping, and 3) airway smooth muscle thickening was not significantly associated with any functional abnormality.

**Comments on Methodology**

Subjects were not selected on the basis of a functional diagnosis, but they were included consecutively provided that the extension of the tumor to be removed...
was not such that it was likely to interfere with measurements of overall lung function. This allowed us to obtain a group with a continuum of lung function ranging from normality to overt airway obstruction. Owing to the impossibility of making a longitudinal study of this nature, i.e., based on the analysis of surgical specimens, this approach seems the only one available to correlate changes in structure and function in the transition from normal to abnormal lung function.

Only small airways (<2 mm diameter) were analyzed. Therefore, any relationship between changes in large airways and lung function cannot be evaluated. This limitation does not seem to invalidate this study, as chronic airway obstruction developing in nonasthmatic smokers is probably related to changes first occurring in the peripheral airways (15, 26).

Flow-volume curves were obtained by plotting flow against mouth volume, thus not taking into account thoracic gas compression. Greater gas compression during maximal than partial expiratory maneuvers might account for MP_{50} values <1, and this effect should be greater in subjects with lung hyperinflation. However, it seems unlikely that this mechanism was responsible for the effect of deep inhalation observed in this study for at least three reasons. First, in asthmatic subjects, gas compression of maximal expiratory maneuvers was found equal to (28) or only slightly greater than (9) that of partial expiratory maneuvers. Second, in asthmatic subjects, the effects of deep inhalation on airway caliber are in the same direction when assessed by forced expiratory flows or airway conductance (30, 32), which is minimally or not at all affected by gas compression. Third, in subjects with established COPD, no correlation was found between maximal-to-partial flow ratio and the common indexes of lung hyperinflation, namely, functional residual capacity and TLC as percent of predicted (5).

Comments on Results

Previous structure-to-function studies have shown that airflow obstruction (3, 39) and airway hyperresponsiveness (10, 34) in COPD subjects are related to thickening of airway walls, particularly in their inner (mucosal) layer. This suggests that, for a given airway smooth muscle linear length, the airway caliber is less than in normal airways for merely geometric reasons (18, 25). The results of the present study confirm these previous findings and theoretical predictions, as both the ratio FEV\textsubscript{1}/FVC and airway reactivity (slope of the MCh dose-response curve) were significantly correlated with the thickness of total airway wall but not of airway smooth muscle. The significant correlation between airway caliber and PD_{20}, in those subjects in whom it could be calculated, likely reflects the relationship between flow and radius of airway lumen, thus further supporting a major role for geometric factors in determining airway responsiveness in nonasthmatic subjects. These data seem, therefore, to support the opinion that smooth muscle plays only a minor role in airway narrowing in nonasthmatic subjects (19). This conclusion, however, would be based on the unproven assumption that the force developed by the airway smooth muscle is proportional to its mass. Indeed, a recent report suggests that the airway smooth muscle of COPD subjects may have an increased ability to generate force, which correlates with the degree of airflow obstruction (27).

In previous studies (10, 39), the effects of airway-to-parenchyma uncoupling were inferred from the thickness of the outer (adventitial) layer of the airway wall, but this was found to be unrelated to either airflow obstruction (39) or airway hyperresponsiveness (10). These findings may appear surprising as uncoupling of airways from the surrounding lung parenchyma should decrease the load on airway smooth muscle, which would then accommodate at a shorter than normal length for any given degree of activation (21). There are, however, reasons for these data. First, the FEV\textsubscript{1}/FVC may not reflect the real degree of airway obstruction when RV is increased. Second, an increase in airway responsiveness, as inferred from the FEV\textsubscript{1} PD_{20}, may reflect an increase in airway smooth muscle constriction more than a decrease in elastic load (14).

Third, thickening of the outer wall layer may not directly reflect airway-to-parenchymal uncoupling, as it does not necessarily mean less force transmission. In the present study, the number and the integrity of alveolar attachments to the airway wall were used, which may represent a more direct estimate of the coupling between airways and surrounding lung parenchyma (36). Moreover, the reduction of FVC during MCh-induced bronchoconstriction was used as an index of air trapping, which is more likely to occur as a result of closure or extreme flow limitation in relatively small airways. However, the occurrence of air trapping (FVC/FEV\textsubscript{1} slope) during induced bronchoconstriction was significantly correlated with the airway wall thickness but unrelated to the number (AA\textsubscript{i}) or the integrity (AA\textsubscript{d}) of alveolar attachments. This finding does not disprove that airway-to-parenchymal interdependence is an important factor modulating airway narrowing, but it may suggest that its effect is masked by the predominant effect of total airway wall thickening.

Loss of alveolar attachments was significantly associated with a bronchoconstrictor effect of deep inhalation, as inferred from the maximal-to-partial flow ratio. A decrease in airway caliber after a deep inhalation has been reported to occur both in chronic asthma (20, 41) and COPD (8, 29). The mechanisms underlying this phenomenon are poorly understood, and to the authors’ best knowledge no studies attempted to relate it to airway or parenchymal morphology. According to the original hypothesis of Froeb and Mead (13), a transient reduction of airway caliber would follow a deep inhalation to TLC if parenchymal hysteresis exceeds airway hysteresis. There are, however, other mechanisms that can explain the reduction of forced expiratory flow after a deep inhalation. Inhomogeneous emptying of lung may cause forced expiratory flow at low lung volume to be less on maximal than partial flow-volume.
curves, mainly due to contributions by slow-emptying units (23). It seems, however, unlikely that inhomogeneous emptying could account for the present data for two reasons. First, the M/P_{40} ratio was on average <1 and not significantly different in obstructed and in nonobstructed subjects, who are likely to have different degrees of lung inhomogeneity. Second, in 10 subjects the M/P_{40} was measured after MCh and in 8 of them it was found to be increased (P = 0.059, Wilcoxon’s matched-pairs test) with bronchoconstriction, which is likely to be associated with an increase rather than a decrease of lung inhomogeneity. Another mechanism by which deep inhalation may cause bronchoconstriction is the development of active force by the airway smooth muscle. This may be the result of a myogenic response to stretch (22, 31, 38) or a reflex response to constrictor mediators released during stretching. Active bronchoconstriction, however, takes several seconds to develop and is detected by serial measurements of airway conductance but not by M/P_{40} (31).

Although significant correlations cannot be taken as evidence of causal relationships, the findings of the present study suggest that a bronchoconstrictor effect of deep inhalation may be in some way related to a loss of peribronchial alveolar attachments. There are several ways by which loss of alveolar attachments may result in a transient airway narrowing. First, loss of alveolar attachments is likely associated with a reduced tidal stretching of airway smooth muscle. This, in turn, may increase airway wall stiffness by different, yet not mutually exclusive, mechanisms. Shen et al. (37) proposed that continuous cyclic stretching of airway smooth muscle decreases its tone by maintaining the cytoskeleton in a less force-developing arrangement; Fredberg et al. (11) proposed that reduced tidal stretching may facilitate the latch state of airway smooth muscle, which is mechanically characterized by an increased stiffness and less hysteresivity (12). Therefore, if the lung dilates normally during deep inhalation while the stiff (less hysteretic) airways do not, then the distending pressure around them will be less and so will be their caliber during the next expiration. Using a sensitive imaging technique in dogs with moderately increased airway smooth muscle tone by continuous MCh intravenous infusion, Mitzner and Brown (24) found that minimizing tidal stresses resulted in a bronchoconstrictor response to deep inhalation, and this was associated with a reduction in lung volume. They suggested that the parallel reductions in airway caliber and lung volume after deep inhalation may reflect an active force developed by the airway smooth muscle when stimulated by a deep inhalation taken after a period of reduced tidal stretching. Whether this mechanism may also occur in noncontracted lung is, however, unknown. Finally, in the presence of reduced radial forces on airway walls, the airway caliber may decrease at full lung inflation because of longitudinal traction (4) and remain smaller for a while during the next exhalation. This mechanism is, however, observed only in subjects with severe emphysema, whereas a decrease in airway caliber following deep inhalation is observed in the majority of subjects in the present study without significant difference between those with and those without airflow obstruction.

In conclusion, the results of the present study suggest that airway wall thickening is a major mechanism for airway closure to occur in smokers. Furthermore, loss of alveolar attachments to airway walls may contribute to the development of a bronchoconstrictor effect of deep inhalation.

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