Altered airway surfactant phospholipid composition and reduced lung function in asthma

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Altered airway surfactant phospholipid composition and reduced lung function in asthma. J Appl Physiol 89: 1283–1292, 2000.—Pulmonary surfactant in bronchoalveolar lavage fluid (BALF) and induced sputum from adults with stable asthma (n = 36) and healthy controls (n = 12) was analyzed for phospholipid and protein compositions and function. Asthmatic subjects were graded as mild, moderate, or severe. Phospholipid compositions of BALF and sputum from control subjects were similar and characteristic of surfactant. For asthmatic subjects, the proportion of dipalmitoyl phosphatidylcholine (16:0/16:0PC), the major phospholipid in surfactant, decreased in sputum (P < 0.05) but not in BALF.1 In BALF, mole percent 16:0/16:0PC correlated with surfactant function measured in a capillary surfactometer, and sputum mole percent 16:0/16:0PC correlated with lung function (forced expiratory volume in 1 s). Neither surfactant protein A nor total protein concentration in either BALF or sputum was altered in asthma. These results suggest altered phospholipid composition and function of airway (sputum) but not alveolar (BALF) surfactant in stable asthma. Such underlying surfactant dysfunction may predispose asthmatic subjects to further surfactant inhibition by proteins or aeroallergens in acute asthma episodes and contribute to airway closure in asthma. Consequently, administration of an appropriate therapeutic surfactant could provide clinical benefit in asthma.

asthma; surfactant; phospholipid; mass spectrometry

PULMONARY SURFACTANT FORMS a layer at the air-liquid interface within the alveoli and airways (12), and functional surfactant, with a phospholipid composition similar to alveolar-derived material, has been isolated from all levels of the porcine bronchial tree (2). The disaturated nature (no carbon:carbon double bond) of the major phospholipid molecule, dipalmitoyl phosphatidylcholine (16:0/16:0PC), enables surfactant to withstand very high surface pressure, and this is thought to prevent collapse of small alveoli and conducting airways (9). In vitro studies have shown that increased amounts of plasma proteins, phospholipases, cell membrane lipids, and fatty acids impair the surface tension-lowering ability of surfactant (11, 14, 18, 19, 34). Such surfactant inactivation may contribute to the pathophysiology of pulmonary diseases, among which particular interest has focused on acute respiratory distress syndrome (25, 33).

Surfactant dysfunction, together with mediator-induced bronchoconstriction and airway edema, may also contribute to the airway obstruction characteristic of asthma. Airway resistance increased and a surfactant dysfunction developed in ovalbumin-immunized guinea pigs challenged with aerosolized ovalbumin, effects that are both prevented and partly reversed by administration of exogenous surfactant (28). In two studies in mice, after exposure to ozone (8) and after infection with respiratory syncytial virus (37), surfactant dysfunction developed in parallel with severe impairment of respiratory function. For human subjects, surface tension function of sputum was reported to deteriorate during an acute attack of asthma, and aerosolized surfactant improved baseline lung function (23, 24). In two recent studies, surfactant function was inhibited after segmental allergen challenge to mildly asthmatic subjects (17, 21). Analysis of the phospholipid composition of bronchoalveolar lavage fluid (BALF) after local allergen challenge, using electrospray ionization mass spectrometry (ESI-MS), revealed a significantly decreased 16:0/16:0PC concen-
tration, which correlated strongly with both the severity of surfactant dysfunction and the increased BALF protein concentration in asthmatic subjects (15). The molecular species distribution of the altered phosphatidylcholine (PC) in BALF after allergen challenge was characteristic of infiltration of plasma lipoprotein.

These observations suggest that surfactant dysfunction in acute exacerbations of asthma may be due to interactions between aggregates of surfactant and infiltrating plasma lipoproteins, in addition to the significant inflammation and leakage of other plasma proteins into the airway lumen. However, it is not clear whether these findings in the acute setting extend to longer term alterations of surfactant composition or whether any such changes contribute to the overall severity in this chronic inflammatory disease.

Consequently, we undertook a comprehensive analysis of lung surfactant phospholipid composition and function in adult asthmatic subjects with varying degrees of disease severity compared with nonasthmatic adult control subjects. We used sputum induction with hypertonic saline to sample predominantly the lining fluid of the proximal airways and compared sputum surfactant composition with that of BALF, which samples the alveoli and more distal bronchial tree. Surfactant phospholipid composition was measured by ESI-MS and quantified as individual molecular species. This enabled us to explore relationships of the various surfactant components in diseased airways, with surfactant function measured by the capillary surfactometer and with baseline lung function assessed by spirometry. Additionally, this detailed phospholipid analysis permitted an evaluation of the relative importance of the various potential mechanisms underlying any alterations to surfactant composition in asthma.

METHODS

Materials

Phospholipid standards and streptavidin-alkaline phosphatase conjugate for ELISA were obtained from Sigma-Aldrich Chemical (Poole, Dorset, UK). HPLC-grade solvents for ESI-MS were supplied by BDH (Poole, Dorset, UK). Filters for processing sputum and BALF samples were obtained from Becton Dickinson (Oxford, UK). Microtiter plates were from ICN Biomedicals (High Wycombe, Bucks, UK). Phosphate-buffered saline (PBS) was from Life Technologies (Paisley, Scotland, UK).

Study Design

There were 23 women and 25 men in the study, with ages ranging from 18 to 61 years. There were 12 control (10 nonatopic and 2 atopic) and 36 asthmatic subjects, characterized as mild (n = 11), moderate (n = 16), or severe (n = 9) using the criteria of the National Heart, Lung, and Blood Institute/World Health Organization workshop (13). Disease severity was assessed on the basis of daytime and nocturnal symptom scores entered into diary cards, with a subjective assessment of wheeze and breathlessness. Other parameters used included the requirement for inhaled short-acting β2-adrenoceptor agonist rescue medication, total daily inhaled and oral corticosteroid use, and twice-daily peak expiratory flow measurements. All patients underwent formal spirometry to determine the forced expiratory volume in 1 s (FEV1) using a Vitalograph dry bellows spirometer, the best of three consecutive measurements being used. Clinical parameters summarized in Table 1 indicate that subjects in both the mild and moderate asthma groups were receiving appropriate medication that provided good control of their symptoms. By contrast, subjects in the severe asthmatic group exhibited poor lung function even when treated with high doses of inhaled steroid and long-term oral prednisolone when clinically indicated (n = 4). The differential leukocyte count in induced sputum showed an elevated proportion of eosinophils, even in the well-controlled asthma groups (Fig. 1).

Each subject underwent flexible fiber optic bronchoscopy and/or hypertonic saline-induced sputum induction, dependent on disease severity. Thirty-one of the subjects (9 control, 22 asthmatic) underwent both bronchoscopy and sputum induction, enabling comparison of surfactant composition in BALF and sputum. Each procedure was separated by at least 1 wk so that the second examination would not be influenced by the first. The study was approved by the Southampton and Southwest Hampshire Local Ethics Committee.

Bronchoscopy and bronchoalveolar lavage. Subjects for fiber optic bronchoscopy (3) were premedicated with 2.5 mg of nebulized salbutamol and 0.5 mg ipratropium bromide, and mild sedation was achieved with intravenous midazolam. Topical anesthesia was achieved with 2% (wt/vol) and 10% (wt/vol) lignocaine applied to the upper respiratory tract and 1% (wt/vol) lignocaine delivered via the bronchoscope to the lower airways to suppress coughing. The bronchoscope (Olympus BF Type XT20, Olympus KeyMed, Southend-on-Sea, Essex, UK) was wedged into a segment of either upper lobe, and bronchoalveolar lavage was performed by instilling 6 × 20 ml aliquots of prewarmed 175 mM saline. Recovered aliquots of BALF were pooled, filtered through a 100-μm cell strainer to remove mucus, and centrifuged (400 g, 4°C, 10 min) to pellet the cellular component. Cytospins were prepared using a Cytospin 2 centrifuge (Shandon Southern Products, Runcorn, Cheshire, UK), stained with May-Grunwald-Giemsa, and 400 cells were counted for the proportion

Table 1. Airway function and inhaled steroid dose for control and mild, moderate, and severe asthmatic subjects

<table>
<thead>
<tr>
<th></th>
<th>Control Group (n = 12)</th>
<th>Mild (n = 11)</th>
<th>Moderate (n = 16)</th>
<th>Severe (n = 9)</th>
</tr>
</thead>
<tbody>
<tr>
<td>FEV1, %predicted</td>
<td>101(89–113)</td>
<td>95(86–117)</td>
<td>95(61–113)</td>
<td>52(25–76)</td>
</tr>
<tr>
<td>Inhaled steroid dose, μg/day</td>
<td>0</td>
<td>0(0–200)</td>
<td>400(0–1,600)</td>
<td>4,000(1,000–4,000)</td>
</tr>
</tbody>
</table>

Values are medians with ranges in parentheses. FEV1, forced expiratory volume in 1 s.
of macrophages, neutrophils, eosinophils, lymphocytes, and epithelial cells. Supernatant aliquots were stored at −20°C for phospholipid analysis.

_Sputum induction._ Sputum induction was performed as previously reported (31). Briefly, subjects inhaled 4.5% (wt/vol) saline administered via ultrasonic nebulizer (DeVilbiss Ultraneb 99, Feltham, Middlesex, UK) for 10 min, with sputum expectoration at 5 and 10 min. The combined sputum fluid phase was diluted fivefold with PBS (29) and processed as described for BALF.

_Preparation of blood neutrophil and plasma._ A neutrophil fraction (≥90% purity) was purified by differential centrifugation over Polymorphprep (Nycod Medium Amersham, Amersham, UK) from an aliquot of blood (10 ml, using lithium heparin as anticoagulant) taken from a control volunteer. An aliquot of plasma was retained for phospholipid analysis.

_Measurement of phospholipid concentration in BAL fluid and sputum samples._ Total phospholipid concentrations in BALF and sputum supernatants were determined as inorganic phosphate (1) after extraction of lipid from 800 μl of BALF or 300 μl of sputum supernatant using chloroform and methanol (4). Phospholipid concentration was calculated by comparison to a standard curve (0–60 nmol) of dimyristoyl phosphatidylcholine (14:0/14:0PC).

_Analysis of phospholipid molecular species by ESI-MS._ Molecular species compositions of phospholipid were analyzed by ESI-MS (15, 32) using a Quattro II triple quadrupole mass spectrometer (Micromass UK, Manchester, UK). Total lipid was extracted (4) from aliquots of BALF and sputum containing 25 nmol of phospholipid, after addition of 14:0/14:0PC (5 nmol) and dimyristoyl phosphatidylglycerol (14:0/14:0PG, 1 nmol) as internal recovery standards. Similar lipid extraction was performed on 5 × 10⁶ neutrophils and 100 μl of plasma, using as internal standard 25 and 50 nmol of 14:0/14:0PC, respectively. BALF, sputum, and plasma phospholipids were analyzed directly on total lipid extracts. Neutrophil phospholipids were fractionated into PC and acidic phospholipid fractions by standard procedures using Bond-Elut aminopropyl sample preparation cartridges (Phenomenex, Macclesfield, UK) (6, 20). Lipid extracts were dissolved in 20 μl of chloroform-methanol (1:2 vol/vol) containing 5 mM NaOH and 5 μl introduced by rhodospray valve injection into a flow of methanol-chloroform-water (80:10:10 vol/vol) pumped at 10 μl/min into the capillary inlet of the mass spectrometer. PC molecular species were detected as sodiated adducts under positive conditions, (M + Na)⁺, whereas phosphatidylglycerol (PG) and phosphatidylinositol (PI) molecular species were detected as their molecular ions under negative conditions, (M–H)⁻.

Spectra were obtained by signal averaging (typically 1 min) and processed by use of Masslynx software (Micromass UK, Manchester, UK). Results were expressed either as a percentage of total phospholipid (the individual sums of PC, PG, and PI molecular species) or as a molar percentage of molecular species within a single phospholipid class. Results were not expressed as absolute concentrations because of inevitable variation in BALF and, especially, sputum recoveries.

Protein analysis. Total protein concentration in BALF was measured with the phenol-Ciocalteau reagent (30).

_ELISA for SP-A._ Native surfactant protein A (SP-A), purified by maltosyl-agarose affinity chromatography (35) from BALF obtained from alveolar proteinosis patients, was used to raise rabbit polyclonal antibodies. These reagents were employed in an ELISA to quantify SP-A in BALF samples, as described previously (32). Results for SP-A were expressed as micrograms per milliliter, with a detection limit of 0.2 μg/ml.

_Measurement of surfactant function._ Surfactant function was assessed with a capillary surfactometer (27). Briefly, the ability of pulmonary surfactant in BALF to prevent airflow closure was evaluated with a glass capillary simulating a terminal conducting airway. The effectiveness of surfactant to maintain airflow through a short, narrow section of the capillary (0.3 mm ID) was measured in response to increased pressure at one end of the capillary. After initial extrusion of liquid, the percentage of the following 120 s that the recorded pressure equalled zero indicated the percentage of time that the capillary was open to free airflow. A sample with maximally well-functioning surfactant registered as “open 100%,” whereas samples with total inability to maintain patency registered as “open 0%.”

BALF had to be concentrated 40 times before being measured on the capillary surfactometer, to values >1.5 mg/ml total phospholipid. An aliquot (1 ml) of the postcell BALF supernatant was centrifuged at 40,000 × g at 4°C for 1 h, and 900 μl of the supernatant was removed. The remaining 100 μl was lyophilized, and 25 μl of the previously obtained high-speed supernatant was added. This procedure concentrated the large aggregate surfactant contained in the pellet ten times by centrifugation and four times by dehydration but also ensured that the concentration of putative inhibitory factors was comparable to that in the original BALF.

_Analysis of Results._

In view of the nonnormal distribution of the data, the Friedman test was used to compare control subjects with mild, moderate, and severe asthmatic groups. If this showed significant differences, the Mann-Whitney U-test was performed for comparison between individual groups of data. BALF and sputum in control subjects were compared using the Wilcoxon signed-ranks test.

RESULTS

_Molecular species and classes of phospholipids._ The composition of phospholipid molecular species in both
BALF and sputum was typical of previous analyses (15, 32). A representative spectrum of BALF PC (Fig. 2A) clearly shows the preponderance of disaturated and then monounsaturated species, whereas BALF PG and PI were composed chiefly of monounsaturated species (Fig. 2D). These surfactant phospholipid compositions were substantially different from those of either cell membranes or plasma. For instance, in contrast to the large concentration of disaturated 16:0/16:0PC in surfactant, the predominant molecular species of neutrophil PC were monounsaturated, with 16:0/18:1PC being the major component (Fig. 2B). By contrast, plasma PC was dominated by the diunsaturated species 16:0/18:2PC, 18:1/18:2PC, and 18:0/18:2PC (Fig. 2C).
Table 2. Phospholipid molecular species composition in paired BALF and sputum supernatant samples from control subjects

<table>
<thead>
<tr>
<th>Molecular species</th>
<th>BALF (m/z)</th>
<th>Sputum (m/z)</th>
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<tbody>
<tr>
<td><strong>PC composition (mol% of total PC)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>16:0/16:0PC (m/z 728)</td>
<td>8.7(7.1–10.5)</td>
<td>7.7(5.6–10.0)</td>
</tr>
<tr>
<td>16:0/16:1PC (m/z 754)</td>
<td>10.4(7.9–13.7)</td>
<td>8.5(6.9–11.0)*</td>
</tr>
<tr>
<td>16:0/16:0PC (m/z 756)</td>
<td>50.0(44.9–56.2)</td>
<td>52.7(47.8–56.7)*</td>
</tr>
<tr>
<td>16:0a/18:1PC (m/z 768)</td>
<td>2.7(1.5–4.4)</td>
<td>2.5(1.5–3.5)</td>
</tr>
<tr>
<td>16:0/18:2PC (m/z 780)</td>
<td>5.7(4.4–8.3)</td>
<td>4.9(4.3–7.3)</td>
</tr>
<tr>
<td>16:0/18:1PC (m/z 782)</td>
<td>10.4(8.9–14.2)</td>
<td>12.4(9.7–14.5)</td>
</tr>
<tr>
<td>16:0/18:0PC (m/z 784)</td>
<td>2.0(1.0–4.1)</td>
<td>2.6(1.5–4.1)</td>
</tr>
<tr>
<td>18:0/18:2PC (m/z 808)</td>
<td>2.4(1.2–2.9)</td>
<td>2.5(1.5–4.1)</td>
</tr>
<tr>
<td>Other</td>
<td>6.8(1.5–11.6)</td>
<td>5.9(1.0–10.0)</td>
</tr>
</tbody>
</table>

| PG composition (mol% of total PG) | | |
| 16:0/16:0PG (m/z 721) | 6.6(4.2–13.3) | 6.7(3.2–10.4) |
| 16:0/16:2PG (m/z 745) | 8.2(6.2–10.2) | 8.3(6.3–18.5) |
| 16:0/18:1PG (m/z 747) | 33.0(22.9–38.3) | 27.8(17.0–34.0)* |
| 18:0/18:1PG (m/z 771) | 5.1(2.7–13.9) | 6.6(3.5–9.5) |
| 18:0/18:2PG (m/z 773) | 22.3(14.7–28.4) | 21.1(15.4–28.0) |
| 18:0/18:1PG (m/z 775) | 20.7(13.7–24.1) | 25.7(18.6–28.3)* |
| Other | 1.9(0–5.7) | 2.5(0.9–11.9) |

| PI composition (mol% of total PI) | | |
| 16:0/16:1PI (m/z 835) | 20.9(9.0–34.9) | 20.7(16.5–25.6) |
| 18:0/18:1PI (m/z 861) | 32.0(21.9–55.4) | 33.7(22.8–47.0) |
| 18:0/18:1PI (m/z 863) | 31.3(14.4–43.6) | 27.7(18.4–45.5) |
| 18:0/20:4PI (m/z 885) | 8.2(4.2–14.8) | 9.3(6.1–14.5) |
| Other | 4.0(0–8.2) | 4.5(2.0–7.2) |

Values are medians (ranges) for mol% composition (n = 12). BALF, bronchoalveolar lavage fluid; PC, phosphatidylcholine; PG, phosphatidylglycerine; PI, phosphatidylinositol. *P < 0.05.

2C). Neutrophil membranes contained negligible PG, and their major PI species was 18:0/20:4PI (Fig. 2E), in contrast to the monounsaturated nature of surfactant PI (Fig. 2D). For this study, the importance of this distinct distribution of molecular species compositions of phospholipids from different tissues was that it provided the basis for the approach to determine mechanisms of altered surfactant phospholipid composition in asthmatic subjects.

Control subjects. The detailed compositions of the major species of PC, PG, and PI from BALF and sputum are compared in Table 2. ESI-MS resolved the same eight species of PC, six species of PG, and four species of PI in all samples. There was a clear relationship between the PC compositions of these two sample types collected from the same individuals. The grouped data showed that sputum contained slightly more 16:0/16:0PC and less 16:0/16:1PC than BALF, and there was a positive correlation between the mole percent (mol%) of 16:0/16:0PC measured in BALF and in sputum from the same subjects (r = 0.891, n = 9, P = 0.011). Because sputum and BALF were collected at least 1 wk apart, these results suggested that the fractional concentration of 16:0/16:0PC in lung surfactant was relatively constant for any individual over time.

Compared with the relatively tight grouping of the PC data, there was considerably greater variability for both PG and PI compositions. Moreover, no PG or PI species correlated between BALF and sputum when compared between individuals. This result suggested that there was less coordination of PG and PI compositions between BALF and sputum. There were consistent differences in PG composition between these two sample types, with the mol% of 16:0/18:1PG being lower in sputum than in BALF, and that of 18:0/18:1PC being higher. There were no significant differences in PI species between BALF and sputum. Finally, there were no differences between the relative proportions of total PC, PG, and PI between BALF and sputum.

Asthmatic subjects. Comparable analyses of BALF and sputum from asthmatic subjects resolved the same number and identities of PC, PG, and PI molecular species reported above for control subjects. Compared with control subjects, there were no significant differences to the relative concentrations of total PC, PG, or PI (Table 3) or to the molecular species of PG and PI (results not shown) for any of the asthmatic groups either in BALF or in sputum. Similarly, although there was a trend for 16:0/16:0PC in BALF to decrease in the asthmatic groups, this was not significant compared with the control subjects (Fig. 3A). In contrast, the mol% of 16:0/16:0PC in induced sputum was significantly lower in the mild, moderate, and severe asthmatic compared with control subjects (Fig. 3B). The variation in sputum 16:0/16:0PC increased progressively with disease severity, with maximum values remaining constant between groups.

Mechanisms of altered sputum PC composition. The decreased mol% of 16:0/16:0PC in sputum from the asthma groups could be due to increased contamination of surfactant with phospholipid from other sources. Because the phospholipid molecular species compositions of inflammatory cell membranes and plasma lipoproteins were very different from those of surfactant (Fig. 2), detailed analysis of phospholipid

Table 3. Phospholipid classes compositions in BALF and sputum supernatants from mild, moderate, and severe asthmatic subjects

<table>
<thead>
<tr>
<th>Phospholipid Class</th>
<th>Control BALF (n = 12)</th>
<th>Sputum (n = 12)</th>
<th>BALF (n = 10)</th>
<th>Sputum (n = 11)</th>
<th>BALF (n = 12)</th>
<th>Sputum (n = 16)</th>
<th>Severe Asthmatic Sputum (n = 9)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total PC</td>
<td>77.1(67.7–80.0)</td>
<td>79.7(72.0–84.0)</td>
<td>76.1(70.4–81.0)</td>
<td>79.2(74.5–85.0)</td>
<td>76.5(67.4–87.7)</td>
<td>78.6(66.9–91.4)</td>
<td>80.2(75.0–84.6)</td>
</tr>
<tr>
<td>Total PG</td>
<td>13.9(8.9–18.6)</td>
<td>11.3(10.3–16.6)</td>
<td>18.1(13.3–22.7)</td>
<td>13.0(8.6–17.2)</td>
<td>16.6(9.6–26.8)</td>
<td>12.9(4.8–19.2)</td>
<td>11.9(8.6–15.3)</td>
</tr>
<tr>
<td>Total PI</td>
<td>8.7(4.4–13.5)</td>
<td>9.1(5.0–12.4)</td>
<td>8.1(4.4–14.7)</td>
<td>6.2(3.6–12.5)</td>
<td>5.9(3.1–11.6)</td>
<td>8.1(3.9–15.4)</td>
<td>7.0(4.4–10.7)</td>
</tr>
</tbody>
</table>

Results are fractional concentrations (medians with ranges indicated) of the sum of concentrations of all molecular species of PC, PG, and PI.
profiles was used to provide information about the sources and extent of any such contamination. Results presented in Fig. 4 include all subjects to avoid bias due to selection of patients with more severe inflammation, but essentially identical correlations were observed if the analysis was restricted to subjects with asthma.

The decreased 16:0/16:0PC in sputum did not correlate with the other disaturated species 16:0/14:0PC, which is integral to surfactant but is a minimal component of either cell membrane or plasma PC (Fig. 4A). In contrast, there was a strong negative correlation between 16:0/16:0PC in sputum and 16:0/18:2PC (Fig. 4B, r = −0.713, P < 0.001), the major species of plasma PC. There was no comparable correlation between sputum 16:0/16:0PC and either 16:0/18:1PC (Fig. 4C), the major species characteristic of cell membrane PC, or 18:0/20:4PI (Fig. 4D), the predominant component of inflammatory cell PI. Caution must be exercised in the interpretation of results presented in percentage terms, as decreases in one component must, by definition, be accompanied by compensatory increases in others. However, these results are consistent with altered sputum PC composition in asthma being due at least in part to infiltration of soluble lipoprotein com-

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**Fig. 3.** Concentration of 16:0/16:0PC (mol% of total PC) in BALF (A) and sputum (B) from control and asthmatic subjects. *P < 0.05, **P < 0.01.

**Fig. 4.** Correlations in sputum of 16:0/16:0PC with 16:0/14:0PC (palmitoyl myristoyl PC; A), 16:0/18:2PC (palmitoyl linoleoyl PC; B), 16:0/18:1PC (palmitoyl oleyl PC; C), and 18:0/20:4PI (stearoyl arachidonoyl PI; D). All results are expressed as mol% of the respective phospholipid class (PC or PI).
ponents of plasma rather than contamination by cellular membrane material.

**Protein concentration in BALF.** Total protein concentration (median, range) in BALF from control subjects was 68.1 (26.8–113.5) mg/ml and was not significantly increased in either mild asthmatic (84.2, 50.8–248.1 mg/ml) or moderate asthmatic subjects (74.8, 37.5–177.5 mg/ml). No subject exhibited an elevated BALF protein concentration comparable to that reported for asthmatic subjects after allergen challenge (23).

**Concentration of SP-A.** There was no difference in either the SP-A concentration or the ratio of SP-A to 16:0/16:0PC between asthmatic and control groups for either BALF or sputum samples (Table 4). Because the absolute concentration of 16:0/16:0PC was highly variable (ranges for BAL fluid and sputum were 3–76 and 1–164 nmol/ml, respectively), the ratio of SP-A to 16:0/16:0PC was calculated in an attempt to correct for variable sample recovery. Although expression of results as this ratio provided no greater discrimination between asthmatic and control groups, it clearly showed that there was no relative deficiency of SP-A in sputum compared with BALF.

**Surfactant and lung function.** The surfactant function of BALF measured with the capillary surfactometer tended to deteriorate with increasing asthma severity. Median values (and ranges) expressed as “open in %” were 94.7 (4.6–98.4) in the control group, 96.1 (8.7–99.1) in the mildly asthmatic subjects, falling to 71.9 (11.2–99.1) in the moderate asthmatic group, but these differences did not reach statistical significance. Surfactant function of BAL fluid could not be measured for patients with severe asthma because concerns for safety precluded performing bronchoscopy.

The correlation of surfactant function (open in %) with 16:0/16:0PC in BALF fluid (Fig. 5A) showed that, despite a wide variability of results, no individual with a high value of 16:0/16:0PC exhibited poor surfactant function. Similarly, the weak inverse correlation between surfactant function and protein concentration in BALF (Fig. 5B) suggested that individuals with good surfactant function tended not to have an increased protein content of BALF. However, these results provide no substantive evidence to support the concept that increased protein infiltration into the airways is a major determinant of poor surfactant function in stable asthma.

Evaluation of any relationship between lung function and surfactant phospholipid composition proved difficult, largely because lung function was only impaired for patients with more severe asthma. Consequently, there was no correlation between the mol% of 16:0/16:0PC in BALF and FEV1 (% of predicted value), as no samples from patients with severe asthma were included in this comparison. However, sputum samples were readily obtained from such patients, and there was a weak but significant positive association between the mol% of 16:0/16:0PC in sputum and FEV1.

<table>
<thead>
<tr>
<th>Table 4. SP-A levels and SP-A-to-16:0/16:0PC ratio in control and asthmatic subjects of varying severity</th>
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<tbody>
<tr>
<td><strong>Control</strong></td>
</tr>
<tr>
<td>----------------</td>
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<tr>
<td><strong>BALF</strong></td>
</tr>
<tr>
<td>SP-A, μg/ml</td>
</tr>
<tr>
<td>SP-A:16:0/16:0PC, μg/μmol</td>
</tr>
<tr>
<td><strong>Sputum</strong></td>
</tr>
<tr>
<td>SP-A, μg/ml</td>
</tr>
<tr>
<td>SP-A:16:0/16:0PC, μg/μmol</td>
</tr>
</tbody>
</table>

Values are medians (ranges). SP-A, surfactant protein A.

Fig. 5. Correlation of 16:0/16:0PC (mol% total PC; A) and protein concentration (B) with surfactant function (open in %) in BALF.
DISCUSSION

Previous evidence for a potential role of lung surfactant dysfunction in the pathology of asthma has come from studies of allergen challenge to asthmatic subjects (15, 17, 21) or experimental animal models (26, 28) and a beneficial response to exogenous surfactant therapy in acute asthma (24). The results presented here are the first description of an altered surfactant phospholipid composition in asthmatic subjects who were considered to be receiving appropriate therapy according to the Global Initiative for Asthma guidelines (13) and who were clinically stable over the course of this study.

The validation of sputum induction to obtain safe and reproducible samples of the airway-lining fluid has provided a powerful tool to study mechanisms of asthma. Increasingly, induced sputum is being used in clinical practice and to study responses to treatment. The comparison of BALF with sputum collected from the same subjects clearly demonstrates that the phospholipid composition of sputum supernatant is characteristic of surfactant-derived material (Table 2). Not only were there high concentrations of PC, including 16:0/16:0PC, and PG in these samples, but the minor phospholipids such as 16:0/14:0PC and 16:0/16:1PC were also typical of surfactant (20, 22, 32). Moreover, 18:0/20:4PI, the major PI component of inflammatory and epithelial cells, was low in all samples, indicating relatively little contamination with membrane-derived phospholipid. The conclusion that airway surfactant phospholipids are derived directly from alveolar material (2) was supported by the significant correlation of 16:0/16:0PC concentration (mol%) in BALF and sputum collected at different times from the same control subjects, suggesting that the molecular species composition of secreted alveolar surfactant phospholipid was relatively constant.

The lack of any significant difference in surfactant phospholipid composition in BALF between control and asthma groups suggests that the molecular species composition of surfactant secretion within the alveolus is essentially unaltered in asthma. The suggestion of decreased 16:0/16:0PC in BALF was probably due to bronchoscopy sampling a mixture of airway as well as alveolar material. Consequently, additional factors within the airways must have contributed to the significant decrease of 16:0/16:0PC in sputum from asthmatic subjects (Fig. 3B). One suggested explanatory mechanism is phospholipid hydrolysis by increased secretory phospholipase A2 (sPLA2) in BALF from asthmatic subjects after inhaled antigen challenge (5, 7). However, our evidence does not support such a mechanism operating in stable asthmatic subjects under baseline conditions. sPLA2 exhibits a preference for binding and hydrolysis of acidic phospholipids, and there was no decreased total PG in sputum from asthmatic compared with control subjects (Table 3). Moreover, sPLA2 has no selectivity for hydrolysis of 16:0/16:0PC compared with other PC species (6), and, consequently, increased sPLA2 activity would not be consistent with the decreased 16:0/16:0PC in sputum from asthmatic subjects.

Given that the phospholipid compositional analysis supports neither altered synthesis nor increased hydrolysis of surfactant phospholipid, increased contribution(s) of PC from nonsurfactant sources is the most probable explanation for the decreased sputum 16:0/16:0PC in asthma. In this respect, the comparative results presented in Fig. 4 demonstrate the value of detailed phospholipid molecular species analysis by ESI-MS. Because there were distinct distributions of both PC and acidic phospholipid species extracted from different tissue sources (Fig. 2), their determination permitted the source of this nonsurfactant PC in the asthmatic subjects to be evaluated. The correlations of mol% of 16:0/16:0PC with those of 16:0/18:1PC (Fig. 4C) and 18:0/20:4PI (Fig. 4D) clearly showed that there was little contamination of cellular-derived material in sputum. By comparison, the strong negative correlations of mol% 16:0/16:0PC with 16:0/18:2PC (Fig. 4C) and 18:0/20:4PI (Fig. 4D) clearly showed that there was little contamination of cellular-derived material in sputum. By comparison, the strong negative correlations of mol% 16:0/16:0PC with 16:0/18:2PC (Fig. 4B) suggests that infiltration of the airway lumen with plasma lipoprotein material was a major factor in the altered composition of sputum PC in the asthmatic subjects. This appeared to reflect the more considerable lipoprotein infiltration of BALF 24 h after local allergen challenge (15) and suggested an ongoing process at a relatively reduced level.

In contrast with a report from one other laboratory (36), we found no difference in SP-A levels between control and asthmatic groups when measured immunochromically. It is, however, possible that the functional properties of SP-A may differ between groups (16), and preliminary studies from our group have suggested a trend for the increased appearance of lower-sized oligomers of SP-A in BALF from the asthmatic group. Such lower-sized oligomers of SP-A could potentially be caused by allelic variants (10), and this may warrant further study.

The decreased 16:0/16:0PC in sputum from asthmatic subjects who are clinically stable (Fig. 3B) sug-

![Fig. 6. Correlation of 16:0/16:0PC (mol% total PC) in sputum with forced expiratory volume in 1 s (FEV1; % of predicted value).](http://jap.physiology.org/Downloadedfrom)
gests that dilution with plasma phospholipid was associated with dysfunction of airway surfactant. It is possible that this background surfactant dysfunction, in addition to the increased concentration of water-soluble surfactant inhibitors, may contribute to the magnitude of the acute asthmatic response. Surfactant inactivation in relatively larger airways could result in increased airway edema and liquid filling, which would contribute to the formation of mucus plugs. This would be reflected in the FEV₁ measurement. In noncartilaginous small airways, for which there is, as yet, no direct evidence, it may also cause airway collapse and subsequent air trapping.

A beneficial effect of surfactant therapy has been demonstrated in reversing airflow resistance and improving lung function parameters in acute animal models of asthma (28) and in humans (24) during acute exacerbations of asthma. Our study provides a basis for surfactant administration being beneficial on both surface activity and lung function in asthma. Because this therapy would be directed toward improving airway function, the dosage regimens needed would be relatively modest when compared, for instance, with those currently used for treating acute respiratory distress syndrome with exogenous surfactant.

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