Surfactant phospholipid changes after antigen challenge: a role for phosphatidylglycerol in dysfunction

R. Duncan Hite,1 Michael C. Seeds,1,2 David L. Bowton,1,3 Bonnie L. Grier,1 Anca M. Safta,1 Rajesh Balkrishnan,1,4 B. Moseley Waite,1,5 and David A. Bass1,2

1Department of Internal Medicine/Sections of Pulmonary and Critical Care Medicine and 2Molecular Medicine, Departments of 3Anesthesiology, 4Public Health Sciences, and 5Biochemistry, Wake Forest University School of Medicine, Winston-Salem, North Carolina

Submitted 19 July 2004; accepted in final form 30 August 2004

Although the importance of surfactant in the physiology of conducting airways was first reported approximately 30 years ago, acceptance of this principle has occurred only recently (26). Multiple structural and animal models have described the importance of a surfactant film to provide surface tension-lowering activity and maintenance of airflow within the conducting airways (12). The relative role of surfactant dysfunction in asthma has been implicated by animal and human models of antigen challenge and by several small clinical trials that suggest surfactant replacement therapy may improve airflow obstruction in acute asthma (2, 24, 25).

Two separate groups have reported surfactant dysfunction in humans after endobronchial antigen challenge (21, 22). Each group identified significant protein changes in the surfactant aggregates (SA), LA/SA ratio. Phosphatidylglycerol (PG) was significantly reduced in the LA of the dysfunctional asthmatic BALs. There was a corresponding significant increase in the ratio of phosphatidylcholine (PC) to PG (PC/PG). These end points were indicative of direct surfactant injury and correlate more strongly with surfactant dysfunction than does protein leak or airway resistance, but the mechanisms for dysfunction are not understood. To test mechanisms that alter surfactant function, atopic asthmatics underwent endobronchial antigen challenge and bronchoalveolar lavage (BAL). BAL fluids were sequentially separated into cells, surfactant, and supernatant, and multiple end points were analyzed. Each end point’s unique relationship to surfactant dysfunction was determined. Our results demonstrate that minimum surface tension (\( \gamma_{\text{min}} \)) of surfactant after antigen challenge was significantly increased with a spectrum of responses that included dysfunction in 6 of 13 asthmatics. Antigen challenge significantly altered the partitioning of surfactant phospholipid measured as a decreased ratio of large surfactant aggregates (LA) to small surfactant aggregates (SA), LA/SA ratio. Phosphatidylglycerol (PG) was significantly reduced in the LA of the dysfunctional asthmatic BALs. There was a corresponding significant increase in the ratio of phosphatidylcholine to PG, which strongly correlated with both increased \( \gamma_{\text{min}} \) and decreased LA/SA. Altered surfactant phospholipid properties correlated with surfactant dysfunction as well or better than either increased eosinophils or protein. Secretory phospholipase activity, measured in vitro, increased after antigen challenge and may explain the decrease in surfactant PG. In summary, alteration of phospholipids, particularly depletion of PG, in the LA of surfactant may be an important mechanism in asthma-associated surfactant dysfunction.

Address for reprint requests and other correspondence: R. Duncan Hite, Sect. of Pulmonary and Critical Care Medicine, Wake Forest Univ. School of Medicine, Medical Center Blvd., Winston-Salem, NC 27157-1054 (E-mail: dhite@wfubmc.edu).

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METHODS

Patient Selection

Subjects with atopic asthma (n = 13) were studied. Each asthmatic met criteria of reversible airflow obstruction demonstrated by an increase in forced expiratory volume in 1 s (FEV1) of ≥15% following β-agonist inhalation, or a methacholine pharmacological challenge test that results in a 20% decrease in FEV1 (PC20) at a dose of <8 mg/ml, and episodic symptoms of including cough, dyspnea, and wheezing (1). Subjects with significant cigarette smoking exposure (>5 pack yr) were excluded. Anti-inflammatory medications including inhaled and systemic corticosteroids, cromones, and leukotriene modifiers were withheld for at least 6 wk before study initiation. Inhaled β-agonists (except salmeterol) were withheld for 6 h, and salmeterol and theophylline were withheld for 24 h before each study. Before enrollment, all subjects completed an informed consent, which was approved by our Institutional Review Board.

Antigen Challenge

To determine the dose used for subsegmental endobronchial challenge, inhaled antigen challenges were performed in each asthmatic subject using cat dander (Felinus domesticus), dust mite (Dermatophagoides farinacea), short ragweed (Greer Laboratories, Lenoir, NC), or standardized cat hair (ALK Laboratories, Wallingford, CT) based on results of prior skin testing. Antigens were administered via a continuous LC Jet+ nebulizer (PARI Respiratory Equipment, Richmond, VA) over 2 min of tidal breathing. The FEV1 was measured at 5-min intervals, and the antigen concentration increased every 15 min until a 20% drop in FEV1 or the highest concentration (1:10) was achieved. Subjects were monitored for a minimum of 10 h following antigen challenge, including determination of late asthmatic response (LAR). All subjects experienced an LAR defined as a drop in FEV1 of at least 15%, which persisted for >30 min. We used 5 ml of a 1:10 dilution of the concentration resulting in a 20% fall in FEV1 for the segmental challenge.

Endobronchial Antigen/Saline Challenge

Bronchoscopy for challenge was performed on day 1 with segmental challenge in either the lingula or right middle lobe using 5 ml of antigen solution described above (and the bronchoscope channel flushed with 5 ml of saline) and 10 ml of saline on the contralateral side. Bronchoscopy was repeated 24 h later (day 2), and bilateral BALs were performed.

BAL

BAL was performed 24 h following endobronchial application of antigen to a single segment of one lung and saline to a single segment of the opposite lung. Fiber-optic bronchoscopy was performed using nebulized and topical Xylocaine with benzoic acid sedation. A single inhalation of isoproterenol (0.131 mg) or albuterol (0.09 mg) was administered via metered-dose inhaler, and then the bronchoscope was introduced transnasally into the lower airways. BAL was performed with three 50-ml aliquots (150 ml total volume) of sterile saline, and BALF was kept on ice until processed (~30 min).

Cell Isolation

The total BALF was measured for volume and centrifuged at 200 g for 15 min. The supernatant was removed and further processed for isolation of surfactant (below). The resulting cell pellet was washed three times with PBS and resuspended in PBS containing 0.1% gelatin. Total cell count and viability were determined by hemacytometer and trypan blue exclusion. Differential cell counts were determined from Cytospin slides (Thermo Shandon, Pittsburg, PA) using Leukostat stain.

Isolation of Surfactant

BALF supernatant (cell free) was centrifuged at 45,000 g for 1 h. Aliquots of the resulting supernatant were stored at ~70°C. The surfactant pellet was washed three times with 100 ml of normal saline (pH 7.4) and resuspended in a known volume of normal saline. Aliquots were stored at ~70°C. LA were defined as those in the pellet after ultracentrifugation, whereas SA were defined as the surfactant remaining in the supernatant (35). The phospholipid content of LA and SA was measured by quantitation of lipid phosphorous using the methods of Bartlett (3). “Total” surfactant recovered represents the sum of LA and SA.

Surfactant Function

A pulsating bubble surfactometer (Electronetics, Amherst, NY) was used to measure surface tension-lowering activity (11). Surfactant from the LA fraction (40 μl) was used at a final concentration of 1.0 mg phospholipid/ml in buffered saline containing 5 mM CaCl2 and was analyzed at 20 cycles/min for 10 min at 37°C in the surfactometer as described previously (20). The results are reported as ymax, which represents the surface tension achieved from the 10-min period.

Protein

The protein content of the cell-free BALF supernatant and the surfactant pellet was measured by the bicinchoninic acid method (Pierce, Rockford, IL), with BSA as standards.

Phospholipid Composition

The phospholipid fraction of samples was extracted via the methods of Bligh and Dyer (4). After extraction, the organic phase was dried under N2, resuspended in chlororom, and analyzed for phospholipid concentration (3). Phospholipid composition of LA was determined by separation of individual phospholipids with high-performance liquid chromatography (HPLC) using a C-18 Kromasil column (Drachrom, Greensboro, NC) and a dual solvent system gradient from 100% Mobile Phase A (CHCl3/MeOH/NH4OH, 80:19.5:0.5) ramped to a maximum of 25% Mobile Phase B (MeOH/H2O/NH4OH, 80:19.5:0.5). Quantitation of individual phospholipid peaks from the HPLC eluent was performed with an evaporative light scatter detector (ELSD; SEDERE, Albertville, France). Retention times and binomial response characteristics of individual phospholipids (PG, PC, phosphatidylglycerol, PI, phosphatidylethanolamine, sphingolipids and lysosphospholipids) were defined with commercial available standards (Avanti Polar Lipids, Alabaster, AL). This sensitive method measures individual phospholipids in the range of 0.1–4.0 nmol and requires a minimum surfactant sample size of 12 nmol. To calculate absolute phospholipid concentrations, a fixed concentration of phosphatidylbutanol was added to a known concentration of sample after lipid extraction to serve as an internal standard.

Phospholipase (PLA2) Activity

Supernatant from each BALF (475 μl) was incubated with Surfanta (Ross Laboratories, St. Louis, MO), which was labeled with either 1H[3H]palmitate-dipalmitoylphosphatidylcholine (DPPC, 4.4 × 10-4 nmol/μl, 0.1 μCi/μl) or 1H[3H]oleate-PG (0.026 nmol lipid/μl, 0.004 μCi/μl) using repetitive vortexing. The 1HPC was purchased from New England Nuclear (Boston, MA). The 1HPC was prepared by TLC purification of PG from membranes of 1H[3H]oleate-labeled Escherichia coli using previously reported methods (23, 33). The phospholipid concentration of the final mixture (500 ml) was 1.0 mg/ml (583.4 nmol of phospholipid/sample). Reaction mixtures were supplemented with CaCl2 (5.0 mM) and incubated at 37°C for 4 h. Lipid extraction was used to stop hydrolysis (4), and the phospholipid concentrations of the samples were determined from Cytospin slides (Thermo Shandon, Pittsburg, PA) using Leukostat stain.
and free fatty acid fractions from the organic phase of the lipid extract were measured as previously reported (20). Hydrolysis is expressed as the percentage of the total radioactivity in the free fatty acid fraction.

Statistics

Data represent means ± SE. Analysis of variance was used to determine correlations between changes in the saline-challenged and antigen-challenged samples. Statistical significance for comparison of subjects with dysfunctional surfactant after antigen challenge and those with normal surfactant function after antigen challenge was determined by paired and unpaired Student’s *t*-tests and Spearman rank correlation.

RESULTS

Antigen Challenge in Asthmatic Population

The differences in BALF cellular response, protein, and surfactant between asthmatics after undergoing segmental endobronchial antigen challenge 24 h earlier and a paired segmental challenge with saline are summarized in Table 1. There was no difference in the volume of BALF recovered between saline-challenged and antigen-challenged samples (54.8 ± 52.8%, n = 13).

Antigen challenge resulted in increased total BAL cells relative to the paired saline-challenged asthmatic samples (124.3 ± 10^4 vs. 34.5 ± 10^4 cells/ml, P < 0.05). All individual cell types analyzed (eosinophils, neutrophils, lymphocytes, and alveolar macrophages) were greater in the antigen-challenged samples, but only the eosinophils were increased significantly (67.8 ± 10^4 vs. 1.2 ± 10^4 cells/ml, P < 0.03). For the entire subject group, eosinophils were increased in 12 of 13 patients, and these cellular changes are consistent with changes reported by Shaver et al. (31) after subsegmental endobronchial antigen challenge of asthma patients.

Increased total BALF protein has been hypothesized by others to be the major mechanism of surfactant dysfunction after antigen challenge (21, 22). Because we measured pulmonary surfactant function using only the phospholipid from the LA fraction in the surfactant pellet resuspended in sterile saline, we measured the protein content of the LA pellet fraction separately from the protein in the BALF supernate. The concentration of protein in the surfactant pellet has been calculated as μg of protein per mg of phospholipids, corresponding to functional studies performed at a phospholipid concentration of 1 mg/ml. As seen in Table 1, protein in the surfactant LA pellet of the antigen-challenged samples was ~50% greater than the saline-challenged samples (315.1 vs. 219.4 μg/mg phospholipids, P < 0.05). BALF supernatant protein was highly variable, such that the increase in the antigen-challenged samples did not reach statistical significance. Thus we confirmed that BALF protein concentrations increased in response to antigen challenge and notably extended that observation to the surfactant LA pellet where function is measured.

Surfactant function was measured as the γmin in the LA pellet using a pulsating bubble surfactometer (Table 1). The antigen-challenged samples demonstrated significantly higher γmin than the paired saline-challenged samples (10.1 vs. 4.4 mN/m, P < 0.05). Table 1 also details the BALF phospholipid recovery, distribution between the LA of the ultracentrifuged pellet and the SA of the remaining supernatant, plus the calculated LA/SA ratio. Total phospholipid recovery (LA + SA) was not significantly different between antigen-challenged and saline-challenged samples. However, the LA/SA ratio was significantly decreased in the antigen-challenged BALFs (2.9 vs. 4.4, P < 0.05). This change in distribution represents a combined decrease in phospholipids in the LA fraction and an increase in phospholipids in the SA fraction, neither of which reached statistical significance separately. In the LA fraction, the two most abundant phospholipids, PC and PG, were decreased but did not reach statistical significance.

Spectrum of Surface Tension-Lowering Activity After Antigen Challenge

Despite a consistent inflammatory response to antigen challenge with increased cells and protein in 12 of 13 patients, surfactant dysfunction as measured by an increased γmin was seen in only 6 of 13 subjects after antigen-challenge between subjects (Fig. 1). The increase in γmin in those subjects with

![Fig. 1. Surfactant function. Asthmatics (n = 13), after endobronchial challenge with saline and antigen, underwent bronchoalveolar lavage (BAL). Surfactant pellets were isolated from BAL and resuspended in buffered saline (1.0 mg phospholipid/ml) with 5.0 mM CaCl2. Minimum surface tension (γmin) was measured with a pulsating bubble surfactometer over 10 min at 37°C. The data represent means ± SE. The dotted line in the antigen-challenged asthmatic results is used to define “dysfunctional surfactant” (≥8.0 mN/m) and “normal surfactant” (<8.0 mN/m). *P < 0.05 comparison of saline- and antigen-challenged observations by ANOVA.](http://ajplung.physiology.org/)
“dysfunctional surfactant” was highly significant (19.6 vs. 2.4 mN/m, P < 0.001 vs. paired saline sample). The remaining seven subjects had no significant change or “normal surfactant” (1.9 vs. 2.4 mN/m). The absolute value of γₘᵢₙ to differentiate between dysfunctional surfactant and normal surfactant function subsets was 8.0 mN/m (Fig. 1), a value determined using the upper 95% confidence limit for the measured γₘᵢₙ in the saline-challenged samples (7.7 mN/m). To test for potential mechanisms that explain surfactant dysfunction, the end points from Table 1 were re-examined with separation of the dysfunctional and normal surfactant asthmatic subgroups.

Comparisons Between the Normal and Dysfunctional Surfactant Groups

**BAL cells.** With antigen challenge, total cells increased significantly within the dysfunctional surfactant subset (192.8 x 10⁴ vs. 28.2 x 10⁴ cells/ml, P < 0.05), shown in Table 2. This intense cellular infiltrate was predominantly characterized by significant increases in eosinophils (114.3 vs. 1.3, P < 0.05). Lymphocytes and alveolar macrophages also increased in the dysfunctional surfactant subset after antigen challenge, but the differences did not reach statistical significance. In the normal surfactant group, there was an increase in total cells and eosinophils (21.3 ± 12.5 vs. 1.2 ± 0.3), but neither change was statistically significant. There were no differences in BALF volume recovery in the dysfunctional and normal surfactant subgroups (not shown).

Because eosinophils have been recently implicated as being important in surfactant dysfunction after antigen challenge, we performed correlation analysis between total eosinophil count and γₘᵢₙ (Fig. 2A) (21). Although the correlation was statistically significant (R = 0.54, P < 0.05), the data were notable for two subjects with significantly increased γₘᵢₙ without a significant change in eosinophils and two subjects with significantly increased eosinophils without a significant change in γₘᵢₙ.

**Protein.** Consistent with the previously discussed hypothesized importance of protein leak in surfactant dysfunction after antigen challenge, protein in the surfactant pellet increased significantly after antigen challenge within the dysfunctional surfactant subset (376.8 vs. 197.6 μg/mg phospholipid, P < 0.05), as shown in Table 2. Protein did not change significantly in the normal surfactant subset group. The correlation of the protein increase on surfactant function after antigen challenge was then examined (Fig. 2B). Similar to the results seen with eosinophils, there was a significant correlation (R = 0.61, P < 0.03), which was slightly stronger than seen with eosinophils, but the R value was heavily influenced by two subjects with very high protein levels. A similar degree of correlation was seen when we compared the BAL supernatant protein with γₘᵢₙ (data not shown).

**Surfactant phospholipid.** The LA/SA ratio was significantly decreased in the dysfunctional surfactant group after antigen challenge (1.3 vs. 4.9, P < 0.001), but not in the normal
Further investigate the PG changes as a component of the total of in vitro monolayers of phospholipid mixtures (10). To PG typically does not explain surface tension-lowering activity protein or eosinophils.

Correlation between the decrease in LA/SA ratio with the increase in $\gamma_{\text{min}}$ was higher than seen for either eosinophils or protein ($R = -0.73, P < 0.005$, Fig. 3), which suggests that changes that affect the distribution of surfactant phospholipid between LA and SA after antigen challenge may provide insight into the mechanism of surfactant dysfunction.

Phospholipid composition. As our primary end point of significance was surfactant function, which was measured on the LA fraction, we primarily focused our analysis on the phospholipid composition from the LA surfactant pellets using HPLC/ELSD. Although the phospholipid content of the SA fraction was not a contributor to surfactant function in our approach, the SA fraction composition was analyzed to completely determine the fate of any changes identified in the LA analysis.

A significant decrease in PG in the LA fraction was seen after antigen challenge in the dysfunctional surfactant group (1.6 vs. 3.4 $\mu$g/ml, $P < 0.05$), whereas PG in the normal surfactant subset was unchanged after antigen challenge (Table 2). The amount of PG recovered was also significantly different on direct comparison of the antigen-challenged dysfunctional surfactant subset to the antigen-challenged normal surfactant subset (1.6 vs. 3.2 $\mu$g/ml, $P < 0.05$). In the total BAL (LA + SA) of the dysfunctional surfactant group, PG decreased slightly but did not reach statistical significance. In the normal surfactant subset, the total PG content increased slightly but also did not reach statistical significance. The reduced PG content in the LA fraction of the antigen-challenged samples correlated with $\gamma_{\text{min}}$ (Fig. 4A) with an $R$ value ($R = -0.63$) that was as high or higher than the $R$ values with protein or eosinophils.

The absolute change in an individual phospholipid such as PG typically does not explain surface tension-lowering activity of in vitro monolayers of phospholipid mixtures (10). To further investigate the PG changes as a component of the total phospholipid pool, we examined the ratio of PC to PG (Table 2). The dysfunctional surfactant group revealed significantly increased PC/PG (7.5 vs. 4.8, $P < 0.05$) after antigen challenge, whereas the PC/PG was unchanged in the normal surfactant group. The increased PC/PG reflected primarily the decrease in PG, since the PC content tended to decrease in both groups. In addition, we compared the relative change in PC/PG between antigen-challenged and saline-challenged samples (calculated by dividing PC/PG of antigen by PC/PG of saline sample, or $\Delta$PC/PG). With this index, the difference between dysfunctional surfactant and normal surfactant subsets was highly significant (1.29 vs. 0.70, $P = 0.001$). Figure 4B reveals the strong correlation between the $\Delta$PC/PG and LA/SA ratio ($R = -0.68, P < 0.005$). The $\Delta$PC/PG index similarly demonstrated a strong correlation ($R = -0.61, P < 0.005$) with an increase in $\gamma_{\text{min}}$ (not shown). Similar indexes for changes in protein and eosinophils between the antigen-challenged and saline-challenged samples did not improve their respective correlation with surfactant function (data not shown).

Interestingly, a decrease in PI, an anionic phospholipid similar to PG, was also seen in the dysfunctional surfactant group vs. the normal surfactant subset after antigen challenge (0.4 vs. 0.8 $\mu$g/ml, $P < 0.02$, not shown).

LPC and LPG were analyzed in the LA and SA fractions of all samples. Only a few samples (pre- and postantigen) were
identified to have any LPC or LPG, and no single sample had more than a trace amount.

**Phospholipase activity.** One potential mechanism that would alter the phospholipid content of surfactant is hydrolysis of surfactant by phospholipases. sPLA$_2$ activity of the BALF supernatant from our samples was measured by hydrolysis of both $[^3]$HPG-labeled and $[^3]$HPC-labeled exogenous surfactant (Fig. 5). Hydrolysis of PG and PC were both significantly greater in the total group of antigen-challenged samples in comparison with their paired saline-challenged samples (PG: 11.6 vs. 4.4%, $P < 0.02$; and PC: 17.3 vs. 6.7%, $P < 0.05$). The increased PLA$_2$ activity after antigen challenge vs. the paired saline samples approached significance in the dysfunctional surfactant subset for hydrolysis of PG (15.3 vs. 3.8, $P = 0.08$) but was virtually unchanged in the normal surfactant subset. The increased PLA$_2$ activity for hydrolysis of PC did not reach significance for either surfactant subset, and the increased activity was approximately equal between the two subsets.

**DISCUSSION**

Our data show significant surfactant dysfunction can result when stable baseline asthmatics undergo subsegmental endobronchial antigen challenge. The surfactant dysfunction covers a spectrum of responses within the total group of asthmatics, but analysis of subjects with significant changes in surfactant function (dysfunctional) vs. those with little or no change (normal surfactant function) highlights the potential mechanisms that may produce surfactant dysfunction. Specifically, we identify a significant decrease in the PG content of surfactant after antigen challenge. This relative drop in PG correlates closely with biophysical properties of the dysfunctional surfactant, seen as an increased $\gamma_{\text{min}}$ and as an altered distribution of surfactant phospholipid reflected in the decreased LA/SA. The decrease in PG may be the result of increased hydrolysis of PG by sPLA$_{25}$ after antigen challenge. These phospholipid changes correlate with surfactant dysfunction better than the numbers of airway eosinophils or increased BALF protein.

Consistent with most antigen-challenged models of asthma, we observed a marked increase in total cells and in eosinophils. The correlation between eosinophils and surfactant dysfunction was modest but significant ($R = 0.54$). However, BALF cell counts may underestimate the role of the eosinophil in the lung inflammation since many eosinophils invade the mucosal tissues of the lung. Eosinophils are a potential source of sPLA$_2$ enzymes that could hydrolyze surfactant, and we have detected four different sPLA$_{25}$ in airway epithelial cells (27, 29).

The small but significant increase in surfactant pellet proteins may be of greater relevance to surfactant dysfunction than the highly variable protein in the BALF supernatants since only the pellet protein was present during measurements of $\gamma_{\text{min}}$ in our methods. Increased surfactant pellet protein was seen only in the dysfunctional surfactant subset. This change in protein is consistent with other reports which showed increased total BALF protein in antigen-challenged asthmatics, suggesting a direct contributing role for plasma-derived proteins in the surfactant dysfunction (17). However, the surfactant LA pellets assayed for $\gamma_{\text{min}}$ in our patient group had relatively small protein concentrations, which would be inconsistent with the nonspecific protein effect on surfactant function seen with proteins such as albumin (>5 mg/ml required) (30). The functional importance of this protein change is further diminished by the weak correlation ($R = 0.61$) between the surfactant pellet protein results and $\gamma_{\text{min}}$. Our results do not exclude the presence of specific proteins, which might be more potent selective inhibitors of surfactant function. It has been previously reported that aggressive washing of surfactant pellets improves surfactant function after antigen challenge (22). Although our samples were washed three times, surfactant dysfunction persisted. Likewise, incubation of exogenous, normal surfactant with BALF supernatants from these patients did not lead to an increase in $\gamma_{\text{min}}$ (data not shown). Although increased protein may contribute to reduced surface tension-lowering ability in asthmatics, it does not solely account for the surfactant dysfunction seen in this study.

The observed changes in surfactant aggregates indicate that the mechanisms of asthma-associated surfactant dysfunction involve direct, though complex, changes in the biophysical properties of the surfactant. Shift of surfactant aggregates from LA to SA may result from enzymatic conversion (e.g., convertase) or from inflammatory processes that target surfactant (9, 36). Although the shift from LA to SA may be the end result of a variety of pathophysiological processes, the addition of serum proteins has not been identified as a mechanism for such shifts. The changes seen in our data include decreased LA after antigen challenge, with a significantly greater decrease seen in the dysfunctional surfactant subgroup than the normal surfactant function subgroup. In addition, an increase in SA is seen...
only in the group with dysfunctional surfactant. These combined changes result in a dramatic decrease in the LA/SA ratio, which correlates well with surfactant dysfunction \( (R = 0.73, P < 0.005) \).

The alterations in phospholipid composition of the surfactant pellets provide additional evidence that asthma-associated surfactant dysfunction is caused, at least in part, by direct injury to surfactant components. The most important alteration in composition is the decrease in PG seen in the dysfunctional surfactant group. The decreased PG is the principal reason for the increase in PC/PG index, which correlates more strongly with surfactant dysfunction and decrease in LA/SA than do the less-specific markers of inflammation, protein and eosinophils. This increase in PC/PG ratio is in contrast to other reports of a decreased PC/PG ratio in asthmatic surfactant. In those reports, an increase in PC was noted, and the increased PC was similar in its fatty acid composition to plasma lipoproteins \( (17) \), which likely reflects the phospholipid composition was measured on the entire BAL including LA and SA fractions. This explanation is further supported by our observation that the PG content of the SA fraction from our samples was not decreased. In our current study, analysis of surfactant function utilized only the LA fraction as did our correlations between function and phospholipid composition. The decreases in PC and PG in our study support the hypothesis that antigen challenge directly causes changes in surfactant lipid composition.

As the second most abundant phospholipid in surfactant, PG performs key functions in adsorption, spreading, and respreading of the phospholipid layer \( (34) \). The interaction of PG, an anionic phospholipid, with the essential hydrophobic surfactant protein, SP-B, may be the central mechanism through which PG performs these functions \( (6) \). Interactions between SP-B and PG play important roles in maintaining the alveolar surfactant layer by enhancing the adsorption of DPPC to the surface after release from the type II pneumocytes and respreading of the monolayer after maximum compression (exhalation) \( (14) \). During maximum compression, PG promotes formation of surface-active pools of phospholipid, which remain just below the surface and are immediately available on re-expansion (inhalation). Furthermore, deficiency of PG has been reported in other models of inflammation-associated airways disease, including the respiratory distress syndromes and cystic fibrosis \( (13, 15, 28) \).

In our subjects, PG and PI are both decreased after antigen challenge in the dysfunctional surfactant subgroup. PI is the second most abundant anionic phospholipid, shares a common synthetic pathway with PG, and serves as a cross-functional phospholipid with PG \( (16) \). The decrease in PI could contribute to surfactant dysfunction in addition to the decrease in PG.

Although many mechanisms might contribute to the changes in phospholipid composition, one direct mechanism is hydrolysis by sPLA\(_2\)\(_5\). Our group has previously demonstrated the presence of increased levels of sPLA\(_2\) in antigen-challenged models of asthma as well as allergic rhinitis \( (5, 32) \). In the current study, we demonstrated markedly increased sPLA\(_2\) hydrolysis of PG and PC in the BAL supernatant from asthmatics after antigen challenge. Although the increase in PG hydrolysis in the dysfunctional surfactant subgroup was greater than in the normal surfactant subgroup, the difference did not achieve statistical significance. Our group has demonstrated that the sPLA\(_2\) family has markedly different activities against the individual surfactant phospholipids \( (20) \). It is possible that multiple sPLA\(_2\)\(_5\) are active in the antigen-challenged BALF and that an sPLA\(_2\) with preference for PG is increased in the dysfunctional surfactant subgroup. The presence of an sPLA\(_2\) with a preference for PG would be more difficult to identify in the presence of hydrolysis from other sPLA\(_2\)\(_5\). Our current data potentially support this possibility, since hydrolysis of PC is increased equally in both surfactant function subsets and could represent activity of an sPLA\(_2\) that is equally active against both phospholipids. The lack of significance in PG hydrolysis in the dysfunctional surfactant group may also indicate that other mechanisms unrelated to sPLA\(_2\) activity may contribute to differences in the phospholipids. For example, differences in synthesis, storage, or release of surfactant phospholipid, in particular PG, in response to an injury or stress such as antigen challenge could also play a role.

Generation of lysophospholipids is typically considered the major pathogenic mechanism for sPLA\(_2\)-mediated abnormalities within phospholipids. In our analysis, we were unable to identify significant increases in either LPC or LPG. However, the absence of lysophospholipids is likely the result of their rapid reuptake by cells within the airway microenvironment, whereby the lysophospholipid is reacylated and used for new phospholipid biosynthesis \( (8) \). Despite the absence of lysophospholipids in the BALF after antigen challenge, the decreased PG observed in the dysfunctional surfactant group suggests sPLA\(_2\) may be an important contributing mechanism. In separate studies, we have demonstrated in vitro that sPLA\(_2\)-mediated surfactant dysfunction is more dependent on the depletion of PG than on the generation of lysophospholipids for several phospholipases \( (19) \).

In summary, our study confirms the importance of surfactant abnormalities in asthma and provides new insights into the possible pathogenic mechanisms. Although our data confirmed the previously reported increase in protein and eosinophils after allergen challenge, our analysis suggests that these are not likely to be sufficient mechanisms to fully explain the functional variability seen within these subjects and that other, surfactant-specific end points are important. Of these end points, an absolute reduction in PG and its subsequent contribution to the relative changes in PC/PG correlated most strongly with surfactant dysfunction. The decreased PG content along with the potentially causative increase in sPLA\(_2\) activity are important observations, as each could represent specific targets for therapeutic manipulation.

Acknowledgments

We gratefully acknowledge the generosity of Dr. Stephen P. Peters (Section on Pulmonary and Critical Care Medicine, Wake Forest University School of Medicine) for review and suggestions regarding this manuscript and of Dr. Robert Dillard (Department of Pediatrics/Neonatology, Wake Forest University School of Medicine) for providing Survanta samples that were used to conduct the phospholipase activity assays.

Some of these results have been previously reported in the form of abstracts.

grants

This research was supported by National Heart, Lung, and Blood Institute Grant PO1 HL-64226 (D. A. Bass) and also in part by the General Clinical Research Center of the Wake Forest University School of Medicine under M01-RR-07122.
DISCLOSURES

Since 1996, R. D. Hite has served on the advisory board for ongoing clinical trials in acute lung injury with an investigational synthetic surfactant, Surfactin (Discovery Laboratories, Inc., Doylestown, PA). During that time, Dr. Hite received stocks as a compensation for his efforts related to that activity. He holds shares of Discovery Laboratories stock (DSCO) and has received direct payments for his consultancy with Discovery Laboratories, Inc.

REFERENCES


