The late asthmatic response is linked with increased surface tension and reduced surfactant protein B in mice

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Haczku, Angela, Elena N. Atochina, Yaniv Tomer, Yang Cao, Colleen Campbell, Seth T. Scanlon, Scott J. Russo, Goran Enhorning, and Michael F. Beers. The late asthmatic response is linked with increased surface tension and reduced surfactant protein B in mice. Am J Physiol Lung Cell Mol Physiol 283: L755–L765, 2002. First published April 26, 2002; 10.1152/ajplung.00062.2002.—Pulmonary surfactant dysfunction may significantly contribute to small airway obstruction during the asthmatic response, but neither its exact role nor its regulation is clear. Surfactant function and composition was studied in an Aspergillus fumigatus (Af)-induced late-phase allergic airway response in sensitized BALB/c mice. The peak of Af-induced airway hyperresponsiveness in sensitized and challenged mice 24 h after allergen provocation coincided with a significant fall in surface activity of the pulmonary surfactant. The underlying changes included time-dependent elaboration of elastin and IL-5 followed by eosinophil influx into the airways. The height of airway inflammation and hyperresponsiveness was preceded by release of IL-4 and marked reductions in surfactant protein (SP)-B, a hydrophobic surfactant protein responsible for maintaining low surface tension of the lining fluid of distal air spaces. Furthermore, intratracheal administration of IL-4 significantly inhibited SP-B, indicating a regulatory role of this cytokine in the surfactant biophysical changes. Thus surfactant dysfunction induced by an IL-4-driven SP-B deficiency after allergen provocation may be an important part of the late asthmatic airway response.

surfactant proteins; asthma; allergy; Aspergillus fumigatus

ALLERGEN CHALLENGES of asthmatic patients lead to a characteristic pattern of an early response that occurs within 15–30 min followed by a second, late-phase reaction that may persist over 24 h (13, 46, 47). The early-phase response of constriction and edema of the airways depends on release of mediators from mast cells and is thought to be a high-affinity IgE receptor-mediated event (5). Inflammation in the late response is characterized by an intricate sequence of influx and activation of inflammatory cells including neutrophils, lymphocytes, and eosinophils, as well as mediator and cytokine release in the airway mucosa (5, 9). This process mimics chronic asthmatic airway changes including altered responsiveness to bronchoconstrictor stimuli. In the late-phase asthmatic response and in chronic airway inflammation, the relationship of the inflammatory components to airway obstruction is still unclear (5, 9, 48).

Pulmonary surfactant is a mixture of phospholipids and proteins that lines the distal air spaces. It contains four unique proteins, surfactant protein (SP)-A, SP-B, SP-C, and SP-D, that are produced by type II pneumocytes. SP-A and SP-D are hydrophilic proteins and play a role in innate immunity (15, 37). SP-B and SP-C, which can be isolated by organic extraction of lung tissue or bronchoalveolar lavage (BAL) surfactant (51, 52), are highly lipophilic proteins with functions in lipid packaging and metabolism and importance in normal lung mechanics and surfactant physiology. Dysfunction of the surfactant system (either a relative deficiency or its inactivation) may play a role in the development and progression of the adult respiratory distress syndrome (21, 53), as well as contributing to the pathogenesis of other inflammatory and infectious lung diseases (3, 4, 7).

Pulmonary surfactant changes may be important in augmenting airflow obstruction in the asthmatic response, since low surface tension is essential in keeping the small conducting airways open (18, 29). After allergen challenge of asthmatic patients and in experimental models of asthma, surfactant phospholipid composition is changed and surface activity is inhibited (19, 30, 33, 38–40, 54), but neither the exact mechanism nor the physiological significance of surfactant dysfunction during the asthmatic airway response is understood.

Previously we showed that repetitive intranasal challenge of sensitized mice with Aspergillus fumigatus (Af) causes a significant fall in the levels of the hydrophobic SP-B and SP-C (24). These results led to

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the hypothesis that impaired production of hydrophobic surfactant proteins contributes to surfactant dysfunction that ultimately leads to increased lung resistance in asthma. To investigate the effects of allergic sensitization and challenge of the airways on surfactant composition and physiology, we characterized a model of the late asthmatic response in BALB/c mice and studied the kinetics of changes induced by a single challenge.

**MATERIALS AND METHODS**

**Murine model of the asthmatic response to Af provocation.** To study the role of surfactant proteins in the allergic response, we used a murine model of Af-induced allergic sensitization. Female wild-type and STAT-6-deficient BALB/c mice were obtained from Jackson Laboratories (Bar Harbor, ME) and were housed under specific pathogen-free conditions. Experiments were performed on mice between 8 and 10 wk of age. All experimental procedures used in this study were under a protocol approved by the Institutional Animal Care and Use Committee of the University of Pennsylvania.

The kinetics of surfactant changes was investigated in a novel model of late-phase response to Af allergenic extract (Bayer Pharmaceuticals, Elkhart, IN). Mice were sensitized by intraperitoneal injections of 20 μg of allergen in 100 μl of PBS solution containing 2 mg of alum (Imject Alum; Pierce, Rockford, IL) on days 0 and 14 and were challenged on day 27 with 25 μl of Af extract in PBS (12.5 mg in 21% glycerol) intranasally. Enhanced pause (Penh) measurements and tissue collection were performed before (0 h) and 1, 6, 12, 24, 48, and 72 h after the intranasal treatment with a separate animal group at each time point. In addition, a group of naive mice received intranasal Af treatment, and a group of sensitized mice was challenged with vehicle (21% glycerol in PBS) and studied 12 or 24 h later. Sensitized mice that did not receive intranasal challenge served as the “0 h” control and were studied on day 27 of the sensitization protocol. Intranasal treatment was performed as described previously (24, 42, 43).

In a separate experiment, STAT-6-deficient mice were sensitized and challenged by the same procedure and were studied 24 h after intranasal provocation together with two groups of sensitized wild-type mice challenged with either Af extract (as positive control) or glycerol (as negative control). Intratracheal injection with recombinant murine IL-4 (PharMingen, San Diego, CA) was carried out after exposure of the trachea in lightly anesthetized mice (50 mg/kg pentobarbital sodium ip) and direct injection of 1.5 μg of IL-4 in 25 μl of PBS via a tuberculin syringe. Controls received PBS alone. Mice were studied 24 h later.

**In vivo measurement of airway responsiveness to intranasal challenge with Af and to inhaled methacholine.** Airway responsiveness to Af and methacholine (MCh) was assessed in conscious, unrestrained, spontaneously breathing mice in a whole body plethysmograph (Buxco Electronics, Troy, NY) (27). The airway function of the animal was determined by a dimensionless parameter: Penh. Penh reflects changes in the wave form of the box pressure signal from both inspiration and expiration and combines it with the timing comparison of early and late expiration (pause)

\[
Penh = \frac{(Te-Tr - 1)}{Pef/Pif}
\]

where Te is expiratory time (the time from the end of inspiration to the start of the next inspiration), Tr is relaxation time (the time of pressure decay to 36% of the total expiratory pressure signal (area under the box pressure signal in expiration), PEF is peak expiratory flow (ml/s), and PIF is peak inspiratory flow (ml/s).

Penh measurements in response to Af were averaged over a 2 x 4-min period. MCh dose responses were generated after baseline readings were taken, and saline inhalation was performed. Increasing concentrations of MCh, between 12.5 and 100 mg/ml, were aerosolized for 1 min each, and responses were measured and averaged over a 4-min period.

**BAL analysis for differential cell count, cytokine, and surfactant content.** Lungs were lavaged with sterile saline (5 ml), and total and differential cell counts were performed as described previously (24–26). To remove cells, we centrifuged the BAL samples at 400 g for 10 min at 4°C. Cytokine levels were determined from cell-free supernate of the BAL by ELISA using antibodies and recombinant cytokines from PharMingen. The cell-free supernate was further separated by a second centrifugation at 20,000 g for 60 min at 4°C to produce the large-aggregate (LA) surfactant fraction (pellet) and the small-aggregate (SA) fraction (supernatant) (3, 4, 24).

Total protein and phospholipid contents of the LA and SA fractions were determined as previously described (3, 4, 24) by the standard methods of Bradford for protein and Bartlett for phospholipid. SDS-PAGE of LA and SA surfactant samples was carried out using NuPAGE 10% Bis-Tris gels (Novex, San Diego, CA) according to instructions of the manufacturer.

Rabbit polyclonal antisera against SP-A, SP-B, and SP-D were obtained, and Western blots were performed as previously described (3, 4, 24). Each lane was loaded with 10 μg of total protein.

**Measurements of surface tension by capillary surfactometry.** The capacity of surfactant to maintain airway patency was measured in a capillary surfactometer that has been demonstrated to simulate terminal human airways as described in detail elsewhere (17). The LA fraction of the BAL was diluted with saline to 1 mg/ml phospholipid concentration. Surfactant preparations (0.5-μl samples) were introduced into the narrow section of a glass capillary and compressed for 120 s, resulting in cyclic extrusion from the narrow end of the capillary permitting airflow and capillary patency. Surfactant dysfunction in the sample results in loss of capillary openness. A microcomputer calculates the percentage of the 120-s study period that the capillary is open to a free airflow. For each sample evaluated, a new capillary is used. Data used here are the means of triplicate assays of a sample.

**ELISA for immunoglobulins.** Serum samples were collected as described before (24–26). Antibodies and recombinant IgE were purchased from PharMingen, and antibody levels were determined according to instructions of the manufacturer. For Af-specific antibody levels, plates (Dynatech, Chantilly, VA) were coated with Af (50 μg/ml in PBS, pH 7.1) and incubated overnight at 4°C. Samples were diluted 1:5 for Af-specific IgE and IgG2a and 1:25 for IgG1 and for total IgE. Data were analyzed with the Microplate Manager software program for the personal computer (Bio-Rad, Hercules, CA).

**Lung tissue histology and analysis of mRNA expression.** After lavage, lungs were inflated with 0.5 ml of paraformaldehyde (4% wt/sodium cacodylate 0.1 M, pH 7.3) and were fixed in the same solution for histological analysis. Paraffin sections prepared from the lungs of naive and sensitized mice were stained with hematoxylin and eosin for evaluation of airway inflammation.

In a different set of experiments, total RNA was isolated from lungs after BAL and specific mRNA content was deter-
mined by Northern blot analysis as described previously (24). Nitrocellulose blots loaded with 10 μg of total RNA per lane were hybridized under high stringency with [γ-32P]cDNA probes for rat SP-A, SP-B, SP-C, and SP-D as previously described (3, 4, 24). The specific signals were normalized for loading by hybridization of each blot with an 32P-end-labeled (γ-32P]ATP 28S rRNA oligonucleotide probe and quantified by phosphorimager (Bio-Rad).

Data analysis. Data were expressed as means ± SE. Time courses and dose responses were compared by ANOVA. To test differences between individual groups, we performed Student’s t-test assuming equal variances. Correlations were investigated by regression analysis. A P value of <0.05 was considered as significant. Data were analyzed with the SigmaStat standard statistical package (Jandel Scientific).

RESULTS

Single challenge of sensitized BALB/c mice with Af induced specific immune and inflammatory changes resembling the human late allergic response. The importance of surfactant changes and their relationship to the sequence of inflammatory events and lung function were assessed in a model mimicking the human early and late allergic response to a single allergen provocation. The time points studied (Fig. 1A) were selected on the basis of the known kinetics of the human allergic response and our previous experience with acute models of locally elicited inflammation in mice (41–49).

Because elevated total and Af-specific IgE and IgG1 are diagnostic of the human disease and may partly be responsible for pathological changes of the lung (8), their levels were monitored during the sensitization period (Fig. 1B) and after Af challenge (Fig. 1C). During sensitization with Af, total serum IgE was closely followed by increases in Af-specific IgG1 (Fig. 1B). Af-specific IgE became significant only after Af provocation. Both the total and Af-specific IgE levels showed biphasic depletion pattern from the serum, with lowest levels 1 and 12 h after intranasal provocation with the Af extract (Fig. 1C).

Morphological evaluation of the lung tissue (Fig. 1D) showed that Af challenge induced a predominantly perivascular and peribronchial inflammatory infiltrate that peaked 12–24 h after challenge (Fig. 1D, top right and bottom left) and largely returned to normal by 72 h (Fig. 1D, bottom right). The photomicrograph at the 12-h time point shows a constricted airway. Such airways were randomly present in tissue samples taken both 12 and 24 h after Af challenge.

We investigated the cellular composition of BAL to determine the mechanisms underlying the airway physiology pulmonary surfactant biophysics. To that end it was particularly significant that intranasal Af challenge induced a sequential influx with a characteristic time-dependent peak of neutrophils and eosinophils into the airways (Fig. 2): neutrophil influx peaked at 12 h (465 ± 126 × 10³ cells) and was followed by eosinophils 12 h later (128 ± 36 × 10³ cells, Fig. 2, A and B). By 72 h, the inflammatory response was largely resolved.

The cell-free supernate of the BAL fluid was analyzed at each time point to assess levels of proinflammatory cytokines including IL-4, IL-5, TNF-α, IFN-γ, and eotaxin. TNF-α release was characteristic at the earliest (1 h) time point that we measured (335 ± 136 pg/ml, not shown), confirming a function in the early allergic processes and the results of previous studies showing surfactant changes 12–24 h after administration of TNF-α (8). Similarly to our previously characterized ovalbumin- and Af-induced models of allergic airway inflammation (24–26, 42), intraperitoneal sensitization and a single intranasal challenge with Af induced local production of a predominantly T helper (Th) 2-type cytokine profile manifested by a significant release of IL-4 (see Fig. 5A), IL-5, and eotaxin (Fig. 2C), but not IFN-γ (not shown). As expected and shown before in a different model of eosinophilic inflammation (41), release of eotaxin at 12 h preceded the peak of eosinophilia 24 h after challenge. IL-5 had a sharp peak at the 24-h time point (Fig. 2C).

Airway hyperresponsiveness to Af and MCh showed a time-dependent association with pulmonary surfactant dysfunction. To assess whether allergen challenge would affect surface activity of the lining fluid of the distal air spaces, we analyzed the LA surfactant fraction of the BAL fluid using the novel technique of capillary surfactometry (17). LA samples of mice sensitized and challenged with Af showed a gradual, time-dependent decrease in percentage of capillary openness, with the lowest values observed in the group 24 h after Af challenge (51.2 ± 0.5% vs. 87.76 ± 8.2% of the 0-h group, P = 0.019, Fig. 3A).

Baseline Penh measurements showed a characteristic, highly significant increase that reached a peak 24 h after challenge of sensitized mice (2.01 ± 0.19 vs. 1.12 ± 0.07) (Fig. 3C). To confirm that this response was allergen specific, we performed additional experiments in which baseline Penh was measured in sensitized mice 24 h after Af challenge and compared with that of sensitized mice that received vehicle (glycerol) challenge (1.25 ± 0.19, P < 0.002), naive (nonsensitized) mice treated intranasally with vehicle (glycerol) alone (Penh = 0.84 ± 0.05) or with Af extract (0.87 ± 0.07) (Fig. 3C).

Airway responsiveness measurements to inhaled MCh were performed 24 h after challenge when the height of inflammatory changes was assumed to occur by airway morphology and Af-specific lung function changes. To compensate for the significant differences we observed in their baseline Penh, we expressed MCh dose responses as percent increases above baseline in each mice, and sensitized and Af-challenged animals were compared with sensitized mice challenged with glycerol alone (ANOVA P = 1.12 × 10⁻⁸, Fig. 3D). These data confirmed an airway smooth muscle hyperresponsiveness to MCh during the peak of the allergic response.

Surface tension changes were related to the conversion of phospholipids between the LA and SA surfactant fractions and a significant fall in the levels of the...
hydrophobic SP-B. To study the hypothesis that the changes in pulmonary surfactant biophysics were caused, at least partly, by alterations in its composition, we analyzed the phospholipid as well as the total protein contents of the LA and SA surfactant fractions. After Af challenge of sensitized mice, there was a time-related phospholipid conversion between the LA and SA BAL fractions, with decreasing LA and increasing SA phospholipids levels: 24 h after Af challenge, the LA phospholipid was 167 ± 14 μg (vs. 227 ± 22 μg
of the 0-h controls), and the SA phospholipid was 103 ± 12 µg (vs. 64 ± 5 µg of the 0-h controls). The peak of the protein levels also occurred 24 h after allergen provocation in each fraction, but it attained statistical significance only in the unfractionated cell-free BAL (1,205 ± 159 vs. 455 ± 22 µg, P = 0.0028).

Individual surfactant protein expression was studied by Western blot analysis in the SA and LA BAL fractions as described before (3, 4, 24). We corrected the densitometric values obtained by Western blot for the total protein amounts in each sample to analyze both the relative and the absolute changes in the surfactant protein levels after challenge. Both the absolute (corrected) and relative (uncorrected) SP-B amounts showed significant, time-dependent reductions in sensitized and challenged mice compared with the 0-h control group. The lowest points of the absolute SP-B content were observed 12 h after provocation (51.1 ± 7.4% of the 0-h controls, P = 0.028) (Fig. 4, A and C). The lowest uncorrected SP-B values were measured at the 24-h time point (39.4 ± 7% of the 0-h controls, P = 0.0008, not shown).

Because hydrophobic surfactant proteins were thought to be important in maintaining proper structural and biophysical characteristics of the pulmonary surfactant, we analyzed the statistical relationship between levels of SP-B and the surface tension of the LA BAL fraction. SP-B levels showed a significant, positive correlation with the percentage of capillary openness (r = 0.55, P = 0.0099; Fig. 4B), indicating that the relative SP-B content (relative to the total protein amounts) may be important in regulating surface tension of the pulmonary surfactant. This hypothesis was confirmed by the strong negative correlation we ob-

Fig. 2. Eosinophil influx into the airways was preceded by significant eotaxin release and paralleled by IL-5 after a single allergen exposure to Af. A: photomicrographs of cytospin preparations of representative bronchoalveolar lavage (BAL) samples of naive and sensitized mice. Cytospins were prepared and stained with Diff-Quik (magnification ×1,000), and total and differential cell counts were made. B: number of BAL eosinophils. Differential cell counting was performed on cytospin preparations by counting at least 100 cells/sample. The absolute number of cells was derived by multiplying the %cell count by the total cell number in each sample. Values represent the total numbers of eosinophils (EP, ●) and neutrophils (NP, □). Data are presented as means ± SE; n = 9–12 in each time point. C: time course of immunoreactive IL-5 and eotaxin in the BAL fluid. Protein release in the BAL was analyzed by ELISA. BAL supernate samples were harvested at indicated time points after single Af challenge. Values are expressed in pg/ml as means ± SE (n = 8–10 for each time point).
served between the percentage of capillary openness and the total protein content of the LA fraction ($r = 0.56, P = 0.0206$; not shown). Such a correlation was not present between the SA protein and percentage of capillary openness (not shown).

**SP-B levels are selectively regulated during the asthmatic response and are not dependent on mRNA transcription.** Levels of the other hydrophobic surfactant protein SP-C were also reduced after allergic sensitization and challenge; however, the values corrected for total LA protein content did not attain statistical significance and followed a somewhat different kinetics, with the lowest point 6 h after intranasal provocation ($69.3 \pm 18.1$, Fig. 4D). Northern blot analysis and densitometric evaluation of the 2.0-kb SP-B and 0.9-kb SP-C bands in the lung tissue revealed marked decreases in the mRNA content (normalized for the 28s mRNA expression) in mice sensitized and challenged with Af compared with unchallenged mice ($50.1 \pm 15.3\%$; $P = 0.045$ and $63.9 \pm 15.5\%$; not significant, respectively) 24 h after Af. Thus the alterations in SP-B mRNA levels occurred $12$ h later than that of the protein decreases, indicating involvement of other mechanisms in addition to mRNA synthesis in regulation of SP-B and SP-C during the allergic response (Fig. 4, C and D). In contrast to the decreases in the hydrophobic protein levels, after sensitization and a single exposure to Af, the hydrophilic SP-A did not change (not shown), and SP-D in the SA surfactant fraction was markedly increased 48 h after Af challenge.
Challenge (403.6 ± 47.1% of the 0-h control). This indicates that hydrophobic and hydrophilic surfactant proteins are differentially regulated during the late allergic response.

**IL-4 release in the BAL fluid coincided with changes in SP-B protein after allergen provocation.** To study the role of IL-4 in altering SP-B production during the allergic response, we evaluated the kinetics of IL-4 release compared with surfactant changes. Notably, levels of IL-4 were increased between the 6- and 24-h time points (with a peak 12 h after **Af** challenge) (Fig. 5A). Statistical analysis of SP-B and IL-4 levels demonstrates that the presence of IL-4 has a significant negative correlation with the SP-B content of the LA BAL fraction (r = −0.7682, P = 0.0094; Fig. 5B).

**IL-4 directly inhibits SP-B production in vivo.** We studied the hypothesis that IL-4 directly affects hydrophobic surfactant protein (SP-B) production by treating BALB/c mice intratracheally with recombinant **IL-4**. Mice were intraperitoneally sensitized with **Af**-alum similarly to the allergen-treated animals as described in MATERIALS AND METHODS. On day 27, however, instead of intranasal **Af** provocation, we administered murine recombinant IL-4 (1.5 g/mouse) in 25 g PBS or PBS alone intratracheally. Compared with the PBS-treated group, IL-4 induced a significant decrease in SP-B in the LA fraction of BAL 24 h later (Fig. 5C).

To investigate whether blocking the effects of IL-4 would reverse the decrease in SP-B and airway hyper-reactiveness we found in mice sensitized and challenged with **Af**, we performed these experiments in STAT-6-deficient mice. STAT-6 has been shown to mediate most of the biological effects of IL-4 (as well as IL-13) (1), and interruption of this signaling pathway is
expected to block the effects that these cytokines exert on allergic airway inflammation. Wild-type BALB/c mice and STAT-6-deficient animals (on BALB/c background) were sensitized and challenged with Af as described. Because the height of inflammatory changes and airway hyperresponsiveness was found to occur 24 h after challenge, we selected this time point to investigate. When compared with wild-type mice, STAT-6-deficient animals displayed significantly attenuated airway responses to Af challenge (2.80 ± 0.25 vs. 1.41 ± 0.14, respectively, \( P = 0.043, n = 6 \) each) that were similar to the responses of glycerol challenged mice (1.37 ± 0.17 \( n = 8 \)) as measured by their Penh values. The SP-B levels in the BAL fluid of STAT-6 knockout mice (126.9 ± 11.7%) were not different from those of glycerol-challenged control mice (100.00 ± 13.5%).

These results confirm a direct regulatory role of IL-4 in hydrophobic surfactant protein deficiency and consequently in impaired surfactant function during allergic inflammation.

**DISCUSSION**

This study demonstrated a time-dependent relationship among surfactant function, lung physiology, and airway inflammation elicited by a single allergen exposure in sensitized mice. We have shown that a significant inhibition of the hydrophobic surfactant protein SP-B correlates with increases in surface tension and raises the possibility that IL-4 plays an underlying regulatory role.

Because the allergic late-phase response may serve as a valuable model for dissecting the inflammatory events of chronic asthma and because the underlying mechanisms of Af sensitization are poorly understood (14), we aimed to recreate this model in mice. This allergen has a particular relevance in our studies on the functional importance and regulation of surfactant proteins in allergic airway response, as surfactant proteins have been shown to be involved in processing of inhaled Af particles (2). In our model, the allergic airway responses elicited by intraperitoneal sensitization and intranasal challenge were clearly Af specific, because there were neither early nor late responses noted after provocation with the vehicle control (glycerol) in sensitized mice. We observed increases in airway responsiveness in longitudinal studies using baseline measurements and by monitoring changes in responsiveness to MCh 24 h after intranasal provocation in a whole body plethysmograph of unrestrained, conscious animals. The late asthmatic response in our murine model displayed similar pathological characteristics to those observed previously in asthmatic patients and other allergen-sensitized animal models (11), as it had time-dependent changes in lung function, in the locally released cytokine profile, and in the influx of inflammatory cells.

The coincidence of airway hyperresponsiveness to Af challenge, as well as to MCh, with increases in pulmonary surface tension 24 h after allergen provocation in our study suggests the possibility that this additional mechanism may significantly modify the caliber of sur-
PULMONARY SURFACTANT IN ASTHMA

Although dysfunction and compositional changes of surfactant in the asthmatic airways have recently been widely noted (10, 17–19, 28–30, 33–36, 38–40, 54), the underlying means seems to remain controversial. Previously proposed mechanisms include infiltration of airways with plasma proteins resulting in altered surfactant phospholipid composition and inhibition of surface activity (28). A role for dysregulation of the hydrophobic surfactant proteins (SP-B and SP-C) has not been raised, although impairment of the intricately regulated synthesis, release, and reuptake of these by type II pneumocytes was shown to result in surfactant dysfunction and is believed to play a major role in disorders of the lung parenchyma such as respiratory distress syndrome (20). On the basis of the findings in this study, we propose the novel concept that surfactant dysfunction observed after allergen provocation of sensitized mice is induced by a deficiency in the hydrophobic SP-B levels.

SP-B is a low-molecular-weight, hydrophobic, surface-active protein that undergoes posttranslational glycosylation and proteolytic processing before packaging in the lamellar body for secretion with phospholipids (22). The importance of this hydrophobic protein to normal lung mechanics and surfactant function is supported by several observations. A well-described clinical syndrome of congenital SP-B deficiency can occur in full-term infants who develop immediate respiratory failure and remain dependent on ventilation with poor lung compliance and hypoxemia (45). All human mutations (6, 16) as well as SP-B knockout mice (12) have similar phenotypic characteristics, including an associated codeficiency of SP-C due to incomplete proteolytic processing of pro-SP-C (50). Finally, monoclonal antibodies against SP-B delivered intratracheally induced respiratory failure in rodents (23).

Inhibition of the hydrophobic surfactant proteins is not unique for allergic airway inflammation, since it was shown in other inflammatory diseases such as respiratory distress syndrome (20) and Pneumocystis carinii pneumonia (3, 4, 7) and was attributed to the regulatory effects of proinflammatory cytokines particularly of TNF-α (8). Although a recent study has described an IL-5-dependent downregulation of the SP-C gene after allergen challenge (44), the role of cytokines essential for development of allergic inflammation remains unclear. Because the majority of the components of a Th2-type inflammation depends on the presence of high levels of IL-4, we hypothesized that this cytokine is at least partly responsible for the inhibition of SP-B levels and consequently for the impaired surfactant function. During the development of the asthmatic response in our model, analysis of the BAL fluid showed a strictly time-restricted release of this cytokine, which coincided with the fall of SP-B levels after allergen challenge. Furthermore, presence of IL-4 in the BAL supernatant showed strong negative correlations with the levels of SP-B, raising the possibility of a negative regulatory relationship during allergen-induced airway inflammation. The direct effect of this cytokine was confirmed by our in vivo experiments in which intratracheal treatment of previously sensitized mice with recombinant IL-4 resulted in significant decreases in SP-B levels in the LA fraction of BAL. In addition, mice lacking STAT-6, an essential signaling molecule that mediates most of the biological actions of IL-4 (1), failed to display reductions in SP-B protein levels after sensitization and challenge with Aff, supporting the importance of IL-4-related pathways in the regulation of this hydrophobic surfactant protein.

The inhibitory effects of IL-4 on SP-B production are supported by a recent article in which Ikegami et al. (31) found that IL-4 transgenic mice show a significant decrease of SP-B mRNA relative to wild-type mice. In a previous report by Jain-Vora et al. (32), these authors also observed loss of SP-B mRNA by in situ hybridization together with reduced immunopositivity for both pro-SP-B and mature SP-B in mice overexpressing IL-4. Seemingly at odds with these results, in the same paper, the authors found a significant increase of SP-B protein levels in lung homogenates and BAL fluid by ELISA in the IL-4 transgenic mice. The explanation for this discrepancy may lie in the nature of inflammatory changes elicited in the IL-4 transgenic mice used in these studies. Unlike our model of a late asthmatic response, in which a single inflammatory event induced a narrow peak of IL-4 release with rapid resolution of changes, long-term local overexpression of IL-4 in the lung of transgenic mice induced complex, age-dependent inflammation, with accumulation of proteins and phospholipids indicating a severe impairment of surfactant metabolism and clearance responsible for dramatically increased surfactant protein levels in the distal air spaces (32).

In summary, we propose that compositional alterations and functional impairment of the pulmonary surfactant are important parts of the allergic airway response. Although the exact mechanisms of regulation and the relevance of our findings to clinical asthma need further clarification, our results suggest that an IL-4-induced inhibition of hydrophobic SP-B may play a major role in surfactant inactivation that, in return, possibly will significantly enhance airway obstruction.

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