

Original article

Surfactant protein levels in bronchoalveolar lavage after segmental allergen challenge in patients with asthma

Background: Allergic asthma is associated with airway inflammation and dysfunction of pulmonary surfactant. Because surfactant proteins (SP) account for immunomodulatory functions as well as biophysical functions, we hypothesized that the allergic response in asthma might be accompanied by a dysregulation of SPs.

Methods: We measured levels of SP-A, SP-B, SP-C and SP-D by enzyme-linked immunosorbent assay in bronchoalveolar lavage (BAL) fluid of 23 asthma patients and 10 healthy control subjects under well-controlled conditions before and 24 h after segmental allergen provocation. These data were related to surfactant function, Th₂ cytokine levels in BAL fluid and to the degree of eosinophilic inflammation.

Results: In patients with asthma, allergen challenge increased BAL levels of SP-B, SP-C and SP-D while SP-A was decreased. For SP-B and SP-D, a moderate increase was also observed after saline challenge. In contrast, no alterations were observed in healthy control subjects. Levels of SP-B and SP-C in asthmatics correlated with the ratio of small to large surfactant aggregates (SA/LA ratio) and correlated negatively with BAL surface activity. Furthermore, increased SP-C but not SP-B levels after allergen challenge correlated with eosinophil numbers, interleukin (IL)-5, and IL-13 in BAL while increased SP-D levels only correlated with eosinophil numbers.

Conclusions: This study demonstrates significant alterations of all SPs in BAL fluid after allergen challenge of which SP-C was most closely related to surfactant dysfunction and the degree of the allergic inflammation.

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There is increasing evidence for a role of surfactant in allergic asthma (1, 2). In previous studies, we and others have demonstrated a dysfunction of surfactant and altered surfactant composition in patients with asthma that underwent local allergen provocation (3, 4). In these patients, surfactant dysfunction correlated with the degree of allergic inflammation. In addition to the alterations of its biophysical functions in asthma, surfactant might also be impaired in its immunological functions.

Surfactant proteins (SP) are known to regulate biophysical surfactant properties as well as immunological surfactant functions. The hydrophobic surfactant proteins SP-B and SP-C are essential constituents of lung surfactant, which are necessary for the surface tension reducing properties and adsorption capacities of the surfactant lipids (5, 6). They are closely associated with the phospholipid-lining layer and promote phospholipid rearrangement and sorting during the breathing cycle (7).

In contrast to SP-B and SP-C, SP-A and SP-D are believed to be molecules of the innate immune system because of their ability to recognize a broad spectrum of ligands through their carbohydrate recognition domains (CRDs). Several studies have shown that SP-A and SP-D

interact with a number of viruses, bacteria and fungi (8). Surfactant protein-A and SP-D might also play an important role in allergic diseases because they interact with inhalable mite allergens (9) and bind to carbohydrate components on allergens from *Aspergillus fumigatus* (10) and grass pollen (11, 12).

Changes of the lung milieu that occur during allergic inflammation might lead to local alterations of SP concentrations. For example, it has been shown that Th₂-type cytokines such as interleukin (IL)-4 and IL-13 which are elevated during the allergic inflammation (13) alter SP-B, SP-C and SP-D expression in animal models (14, 15). So far, there are only few incomplete and inconsistent data on alterations of SP concentrations in patients with allergic asthma (16, 17). Until now, concentrations of SP-B and SP-C in bronchoalveolar lavage (BAL) fluid of asthma patients have not been described.

We hypothesized that SPs become altered during the acute allergic inflammation. Therefore, we measured concentrations of SP-A, SP-B, SP-C and SP-D by enzyme-linked immunosorbent assay (ELISA) in BAL fluid of patients with allergic asthma and healthy controls

under specific inflammatory conditions before and after segmental allergen provocation.

Material and methods

Study subjects

In this study, BAL samples from two studies were analysed (3, 18). The 23 asthma patients (mean age 25.3 ± 0.7 years, 14 males, nine females) had mild intermittent allergic asthma as defined in the GINA workshop report 2002 (<http://www.ginasthma.com>; accessed 26 January 2006) with a history of episodic wheeze with reversible airflow obstruction (FEV_1 : $99.6 \pm 2.6\%$ predicted), and asthma had previously been diagnosed by an independent doctor. Each patient had a positive skin prick test to one or more out of the common allergens [mixed grass pollen, mixed tree pollen, rye pollen, mugwort pollen, ribwort pollen, *Dermatophagoides pteronyssinus*, *D. farinae*, cat fur, dog hair, mixed feather, *Alternaria*, *Cladosporia* from Abelló (Bornheim, Germany) or ALK-Scherax (Hamburg, Germany)]. Bronchial hyperresponsiveness and $PC_{20}FEV_1$ were determined as described (3, 18). Patients were only using β_2 -agonists when required for relief of symptoms. None was treated with corticosteroids, sodium cromoglycate, theophylline or leukotriene antagonists. Mean serum immunoglobulin (Ig)E was 210.1 ± 36.7 kU/l.

The 10 healthy controls (mean age 24.2 ± 1.3 years, seven male, three female) had no history of allergic or other diseases, negative skin prick tests, normal serum IgE (10.7 ± 2.0 kU/l), normal lung function tests (FEV_1 : $109.5 \pm 2.5\%$ predicted) and no bronchial hyperresponsiveness (PC_{20} : >8 mg/ml).

All study subjects were nonsmokers and no subject suffered an acute bronchitis 4 weeks prior to the investigations. All subjects gave their written consent after being fully informed about the purpose and nature of the studies which were approved by the Ethical Committee of Hannover Medical School.

Bronchoscopic procedure with segmental allergen challenge

The bronchoscopic procedures were performed as described previously (3, 18). The bronchoscope (BF 160 P, Olympus Optical, Hamburg, Germany) was wedged into the anterior bronchus of the left lower lobe or inferior bronchus of the lingula and baseline BAL was performed with 6×20 ml sterile saline solution (Baseline). Lavage fluid from the first 20 ml was discarded. The instrument was passed into the superior lingular bronchus and 10 ml saline solution were instilled as a control challenge (Saline). The bronchoscope was

then passed into the anterior segment of the right upper lobe or the medial segment of the middle lobe and segmental allergen challenge was performed by instilling 10 ml allergen solution of either grass pollen mix or *D. pteronyssinus* mix. The allergen extract used for segmental allergen challenge (mixed grass pollen or *D. pteronyssinus*) was that which produced the largest wheal response on skin prick testing and the chosen concentration was one-tenth the dilution in saline that elicited a 3 mm diameter skin wheal response. For instillation, the allergen was diluted in 10 ml of saline solution. In one of the two studies, 10 ml of saline was additionally instilled prior to allergen challenge in 10 patients and five healthy control subjects (18). This did not affect the amount of allergen instilled per segment and did not result in differences in eosinophilic inflammation in the asthmatic subjects ($P = 0.975$). Therefore, the BAL samples of the two studies were analysed together.

After 24 h, all subjects were rebronchoscoped with the same premedication and the two segments that were challenged 24 h before were lavaged.

Processing of BAL cells and BAL fluid

Bronchoalveolar lavage fluid samples were processed as described (18). Briefly, cells were filtered through a 100 μ m filter, centrifuged (300 g, 10 min, 4°C) and the supernatant was aliquoted and stored at -80°C until examination. A differential cell count of BAL cells is provided in Table 1. In asthmatics, there was a decreased BAL fluid recovery after allergen challenge which has been observed in former studies (18). The decreased recovery might be due to the inflammatory response in this segment rather than to the location because the recovery in control subjects was not different (Table 2).

Quantification of SP-A, SP-B, SP-C and SP-D in BAL fluid

Surfactant protein-A in BAL fluid was quantified with a competitive ELISA based on monoclonal anti-SP-A as described previously (19). The hydrophobic surfactant proteins B and C were quantified by solid-phase ELISA systems as described before (20, 21). Surfactant protein-D was detected by using an ELISA protocol described by Griese et al. (22). Determination of SPs was performed on unfractionated BAL fluid after separation of cells.

Determination of total protein, phospholipids and Th₂ cytokines IL-4, IL-5 and IL-13 in BAL fluid

Aliquots of the cell-free supernatant were used for determination of phospholipid concentrations using a standard technique. The assay

Table 1. Bronchoalveolar lavage (BAL) cell counts

Subjects	BALF	Cells ($\times 10^6$)	Eosinophils		Neutrophils		Lymphocytes		Macrophages	
			%	$\times 10^6$	%	$\times 10^6$	%	$\times 10^6$	%	$\times 10^6$
Control	Baseline	6.8 ± 1.0	0	0	1.8 ± 0.4	0.1 ± 0	8.1 ± 0.7	0.5 ± 0.1	89.8 ± 0.9	6.2 ± 0.9
	Saline	15.6 ± 6.8	0	0	$19.4 \pm 5.4^*$	6.0 ± 4.2	6.4 ± 1.0	0.7 ± 0.2	$73.8 \pm 4.9^{**}$	9.0 ± 2.7
	Allergen	27.9 ± 11.5	0.5 ± 0.2	0.1 ± 0	$29.9 \pm 6.2^{**}$	12.5 ± 6.4	6.3 ± 0.9	1.3 ± 0.4	$63.3 \pm 5.7^{**}$	14.0 ± 4.8
Asthma	Baseline	8.3 ± 0.9	1.0 ± 0.3	0.1 ± 0	2.2 ± 0.5	0.2 ± 0.1	10.7 ± 1.7	0.9 ± 0.2	86.1 ± 1.8	7.1 ± 0.8
	Saline	53.7 ± 34.3	0.9 ± 0.2	1.3 ± 1.0	$25.2 \pm 4.9^{**}$	24.6 ± 19.0	7.8 ± 0.9	3.8 ± 2.4	$65.8 \pm 4.7^{**}$	24.0 ± 12.2
	Allergen	$79.7 \pm 26.2^*$	$46.2 \pm 5.8^{*}##$	$45.9 \pm 21.0^{*}#$	$16.0 \pm 3.7^{**}$	17.4 ± 11.3	$5.9 \pm 0.7^{*}##$	$4.0 \pm 1.5^*$	$32.0 \pm 5.0^{*}##$	12.6 ± 3.3

Total cell counts, and percentage of cells in bronchoalveolar lavage fluid from healthy control and asthmatic subjects. BAL fluid was obtained at baseline or 24 h after saline challenge or allergen challenge. Values are given as mean $\pm$ SEM.

* and ** indicate $P < 0.05$ and $P < 0.01$, respectively, compared with baseline values and # and ## indicate $P < 0.05$ and $P < 0.01$, respectively, compared with saline challenge.

Table 2. Bronchoalveolar lavage fluid (BALF) data

Subjects	BALF	Recovery (ml)	Total protein (μg/ml)	IL-5 (pg/ml)	IL-13 (pg/ml)	Phospholipids (μg/ml)	SA/LA ratio	SP-B/PL (%)	SP-C/PL (%)
Control	Baseline	78 ± 3.0	60.5 ± 8.5	15.1 ± 6.0	74.7 ± 14.8	50.0 ± 3.4	0.02 ± 0.01	3.1 ± 0.3	1.4 ± 0.2
	Saline	81 ± 2.0	121.3 ± 46.6	15.9 ± 6.1	97.0 ± 7.5	48.7 ± 7.4	0.15 ± 0.05	3.9 ± 0.8	1.6 ± 0.4
	Allergen	82 ± 3.0	125.0 ± 27.8*	17.3 ± 6.9	93.9 ± 6.7	49.7 ± 4.7	0.07 ± 0.04	4.3 ± 0.7	1.9 ± 0.5
Asthma	Baseline	74 ± 1.7	76.8 ± 8.0	13.1 ± 2.0	83.7 ± 9.6	45.3 ± 3.0	0.10 ± 0.02	2.9 ± 0.2	1.9 ± 0.2
	Saline	73 ± 2.1	128.3 ± 13.9**	13.5 ± 2.2	84.2 ± 11.0	51.5 ± 4.9	0.16 ± 0.04	4.1 ± 0.5*	2.4 ± 0.4
	Allergen	65 ± 4.4*	1366 ± 356.3***##	182.1 ± 72.2*#	132.7 ± 33.7	57.1 ± 7.0	2.74 ± 1.29*#	7.1 ± 1.2**	4.6 ± 0.7***##

Fluid recovery, total protein concentration, levels of interleukin (IL)-5 and IL-13, phospholipid concentration, ratio of small surfactant aggregates (SA) to large surfactant aggregates (LA), and levels of SP-B and SP-C related to phospholipids (PL) in bronchoalveolar lavage fluid from healthy control and asthmatic subjects. BAL fluid was obtained at baseline or 24 h after saline challenge or allergen challenge. Values are given as mean ± SEM.

* and ** indicate $P < 0.05$ and $P < 0.01$, respectively, compared with baseline values and # and ## indicate $P < 0.05$ and $P < 0.01$, respectively, compared with saline challenge.

is based on a phosphorus determination carried out on the lipids extracted with chloroform/methanol as summarized by Hohlfeld et al. (3). Concentrations of IL-4, IL-5 and IL-13 in BAL fluid were analysed by ELISA systems obtained from R&D Systems (Wiesbaden, Germany; 18).

Surfactant aggregate separation and analysis of BAL surfactant activity

To separate surfactant aggregates, the cell-free BAL supernatant was centrifuged at 48 000 *g* for 1 h at 4°C to pellet large surfactant aggregates (LA). The phospholipid contents of the supernatant-containing small surfactant aggregates (SA) and the LA pellet was determined separately as described above.

The surfactant activity of BAL samples was determined with the Capillary Surfactometer (CS; Calmia Medica, Toronto, Canada) as described previously (3).

Statistical analysis

For comparisons of paired data within the study groups the paired *t*-test was used. According to Chinn (23) the *t*-test is preferable to the equivalent nonparametric tests as it is more powerful and robust to non-normality. Correlations were assessed by computing Pearson correlation coefficients. *P*-values of < 0.05 were considered as statistically significant. Unless otherwise stated, the data in the text and figures were expressed as mean ± SEM.

Results

Phospholipid concentration, surfactant activity and levels of IL-4, IL-5 and IL-13 in BAL

The phospholipid concentrations in the cell-free supernatants from healthy subjects and asthmatics were not significantly altered by saline challenge or allergen challenge. As reported previously (3) for a subgroup of asthmatics ($n = 13$) and healthy subjects ($n = 5$), the SA/LA ratio was low at baseline and there was no difference between the two cohorts (Table 2). After allergen challenge, the SA/LA ratio was significantly increased in asthmatics but not in controls. In addition, the surfactant activity determined with the CS ('open in

%)') was significantly reduced after allergen challenge in asthmatics compared with baseline values whereas no significant change was observed in healthy controls.

Interleukin-5 levels in BAL were increased after allergen challenge in asthmatics but remained unchanged in controls. No significant differences were observed for IL-13 levels (Table 2). Interleukin-4 levels were below the detection limit in all samples analysed (data not shown).

BAL surfactant protein levels

The concentration of SP-A in BAL fluid was significantly decreased after allergen challenge in patients with asthma when compared with baseline levels or with levels after saline challenge. In contrast, there was no effect of allergen or saline provocation in the healthy control subjects (Fig. 1A).

Surfactant protein-B concentrations in BAL fluid were significantly increased after saline and after allergen challenge in asthmatic subjects compared with baseline (Fig. 1B). The increase of SP-B after allergen challenge was significantly higher than after saline challenge (Fig. 1B). Relating SP-B levels to phospholipid content basically revealed the same picture except that the difference between saline and allergen was not significant (Table 2).

Regarding SP-C concentrations in BAL fluid, segmental allergen challenge induced a specific increase of SP-C in asthma patients resulting in significant higher values compared with baseline and saline challenge (Fig. 1C). This increase was similar when SP-C levels were related to the phospholipid contents of BAL fluid (Table 2). Saline challenge alone did not affect SP-C concentrations. In the control group, neither saline nor allergen provocation changed SP-B or SP-C concentrations in BAL fluid.

Surfactant protein-D concentrations in BAL fluid were increased after saline and after allergen challenge in the asthma patients whereas no difference was observed in the control group (Fig. 1D).

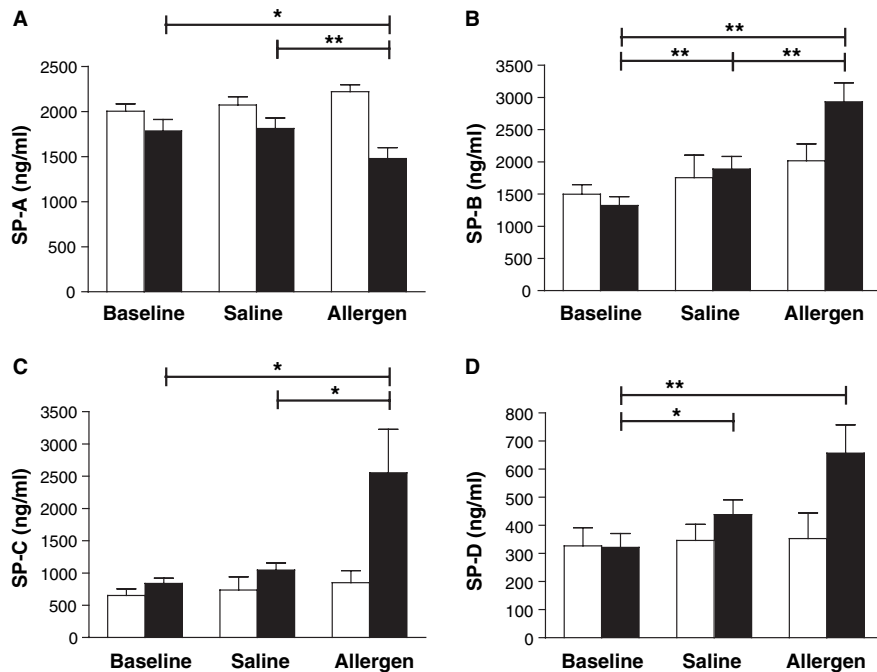


Figure 1. Surfactant protein (SP) levels in bronchoalveolar lavage (BAL) fluid. Concentration of SP-A (A), SP-B (B), SP-C (C) and SP-D (D) in BAL fluid from healthy subjects (open bars, $n = 10$) and patients with asthma (closed bars, $n = 23$) at baseline (Baseline), after saline challenge (Saline) and after allergen challenge (Allergen). * and ** indicates $P < 0.05$ and $P < 0.01$ respectively. Data are given as mean $\pm$ SEM.

Correlation of surfactant protein levels in BAL with eosinophilic inflammation, Th₂ cytokines and surfactant function after allergen challenge

Surfactant protein-C in BAL fluid collected from patients with asthma after allergen challenge correlated with absolute numbers of eosinophils ($r = 0.85$, $P < 0.0001$), with IL-5 ($r = 0.58$, $P = 0.004$) and IL-13 levels ($r = 0.45$, $P = 0.041$). In addition, SP-D in BAL fluid correlated with the percentage ($r = 0.66$, $P = 0.001$) and absolute numbers ($r = 0.52$, $P = 0.002$) of BAL eosinophils. No correlations with variables of allergic inflammation were observed for SP-A or SP-B.

In BAL samples from a subgroup of the asthmatics that were analysed for surfactant function ($n = 13$) both SP-B and SP-C correlated negatively with BAL surface activity expressed as 'open in %' (Fig. 2A,B). In addition, there was a correlation between concentrations of SP-B and SP-C and the SA/LA ratio (Fig. 2C,D). Surfactant protein-D correlated with the SA/LA ratio ($r = 0.57$, $P = 0.0002$) but no correlations with surface activity or SA/LA ratio were observed for SP-A in these samples.

Discussion

We demonstrate significant changes of SP concentrations in BAL fluid of asthmatics after specific induction of an acute allergic inflammation by segmental allergen chal-

lenge. Surfactant protein-B, SP-C and SP-D levels were increased 24 h after allergen challenge while SP-A was decreased. The most pronounced change from baseline was seen for SP-C, and SP-C was most closely related to surfactant dysfunction and the degree of the allergic inflammation.

It is interesting to note that SP changes were seen in the absence of a change in total phospholipids in the cell-free BAL fluid. As reported by Viviano et al. (24), induction of an acute pulmonary inflammation by different stimuli exhibited unique responses of surfactant phospholipid and SP-A to the inflammatory agents. The authors' assumption that activation of the surfactant system is not a universal response to airway inflammation is in line with our observation of altered SP levels and unchanged phospholipids after segmental allergen challenge. Together with our previous findings that BAL total phospholipid levels in asthmatics did not change after segmental allergen provocation (3) and that the altered phosphatidylcholine composition was due to infiltration of plasma phospholipid components (25) we conclude that SP levels in BAL of asthmatics are regulated independently from surfactant phospholipid synthesis and secretion.

Interestingly, we observed decreased SP-A levels while SP-D levels were increased upon allergen provocation. Previous reports on SP-A levels in asthma patients are inconsistent. In a study by van de Graaf et al., decreased concentrations of SP-A in BAL fluid of asthma patients

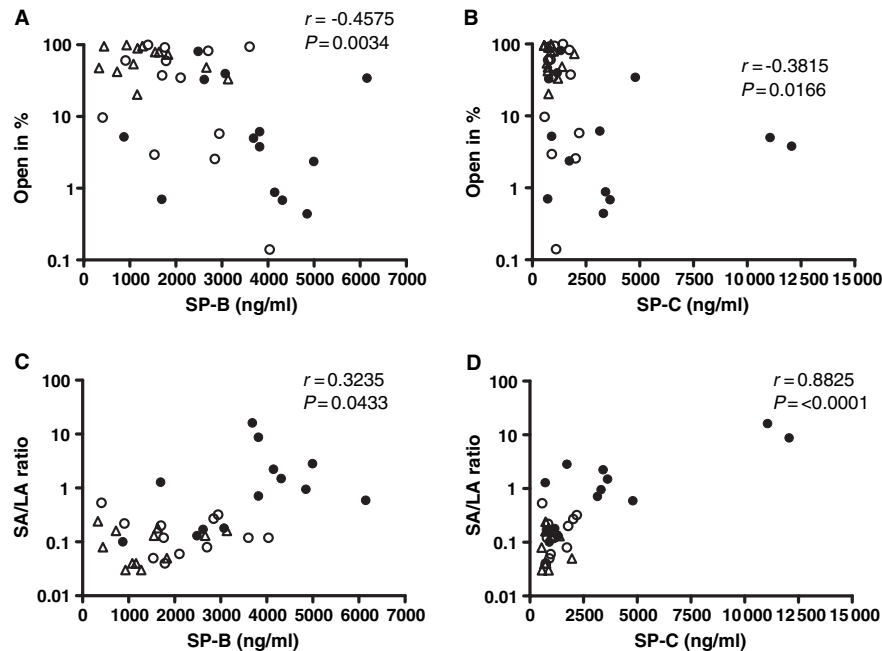


Figure 2. Hydrophobic surfactant proteins correlated with surfactant dysfunction. Negative correlations between surface activity of bronchoalveolar lavage (BAL) fluid evaluated with the Capillary Surfactometer and BAL fluid levels of SP-B (A) and SP-C (B) are shown in the upper panel. Correlations between the SA/LA ratio and BAL fluid levels of SP-B (C) and SP-C (D) are shown in the lower panel. BAL fluid was obtained at baseline (open triangles), after saline challenge (open circles) and after allergen challenge (closed circles) from 13 asthmatics.

compared with healthy controls were observed (16). However, Cheng et al. showed increased levels of SP-A in asthma patients while a third study by Wright et al. did not show any differences between asthma patients and healthy controls (17, 26). As in the latter study, we observed no differences in baseline SP-A levels between mild asthmatics and controls. However, our study has the advantage to show intra-individual changes after the induction of a specific allergic inflammation by segmental allergen challenge that allows the comparison not only to baseline levels but also to levels after control challenge with saline. Thus, we were able to demonstrate that the allergic inflammation, which is characterized by a massive eosinophil influx, resulted in a specific decrease of SP-A compared with baseline and saline challenge. Such a specific decrease of SP-A during the allergic inflammation was already observed in animal studies. In two studies with sensitized BALB/c mice, SP-A levels determined by ELISA, were decreased after allergen challenge (27, 28).

In contrast to decreased SP-A levels, SP-D levels were increased after allergen challenge. This fits well to previous findings as Cheng et al. (17) demonstrated increased levels of SP-D in asthma patients compared with healthy controls. In addition, higher levels of SP-D were consistently found after allergen challenge in animal models (27, 29). In a recent study, Koopmans et al. investigated serum SP-A and SP-D levels before and 24 h after whole lung inhalational allergen challenge in 50 allergic patients (30). In this study, serum SP-D levels

were increased by about 17% compared with baseline while serum SP-A levels were increased by only about 6%. The authors assumed that increased serum levels result from increased permeability of the blood-airspace barrier during the allergic inflammation. In addition, they hypothesized that the pronounced increase in serum SP-D might be due to an increased SP-D production within the lung that is triggered by the allergic inflammation. This view is supported by our observations because we indeed found increased SP-D BAL levels upon allergen challenge. In contrast, decreased SP-A levels in our study would then have to be explained due to leakage into the vascular compartment which is not compensated for by increased SP-A production. To validate this hypothesis is difficult for two reasons. First, we did not measure SP-A or SP-D in serum, but we assume that due to challenge of only one lung segment in our study instead of challenge of the whole lung as performed by Koopmans et al. (30), the effects on SP-A and SP-D in serum might be too low to be detected. Secondly, the hypothesized lack of increased SP-A production in lung cells would have to be demonstrated in histological lung specimens that, unfortunately, cannot be obtained from humans.

Regarding the concentration of SP-B and SP-C, no data exist from asthma patients so far, and no study exists that employed an ELISA technique to determine SP-B and SP-C in asthma animal models. Using Western blot, previous studies showed a decreased protein concentration of SP-B and SP-C in BAL fluid after allergen

challenge in sensitized mice (27, 29, 31). In contrast, we observed a significant increase of SP-B and SP-C in BAL fluid after allergen challenge in asthma patients. Differences between our findings in asthma patients and results from the animal studies might be due to different source material for the SP analysis. In the animal studies, SP-B and SP-C were measured in the LA fractions of surfactant whereas we measured both SPs in whole BAL fluid. We and others (3, 32) demonstrated previously that the LA content in BAL is reduced while the SA content is increased during the allergic inflammation. If only the LA fraction is analysed, a reduced content of the lipid-associated SP-B and SP-C might be observed, although the total concentration of both SPs is increased.

The ELISA used for SP-B and SP-C in our study was previously used to determine SP levels in patients with acute respiratory distress syndrome (ARDS). In these patients, decreased levels of SP-C were observed while levels of SP-B were unchanged (19, 21). These data indicate that the fairly unexpected increase of both SP-B and SP-C after allergen challenge is not an unspecific, but at least for SP-C an allergen-specific response.

Previous studies have shown that SP-B and SP-C play an important role in maintaining biophysical surfactant function and protection against lung injury (33, 34). In our study, increased concentrations of SP-B and SP-C after allergen challenge in asthma patients were correlated with markers of surfactant dysfunction, i.e. increased SA/LA ratios and decreased BAL surface activity. The hydrophobic surfactant proteins SP-B and SP-C become increased upon allergen challenge when surfactant function is disturbed by invading proteins, which might be explained as counterregulation to protect against surfactant dysfunction. However, it has to be kept in mind that the functional integrity of the immunologically quantified proteins was not evaluated. Thus, it might be speculated that SP-B and SP-C, although present at increased concentrations, are functionally inactivated.

The observed alterations of SP concentrations after allergen challenge in asthma patients might be mediated by specific proinflammatory cytokines. For example, Th₂-type cytokines-like IL-4 and IL-13 are elevated during the

allergic inflammation (13). Animal studies with mice transgenic for IL-4 and IL-13 have shown that both cytokines are able to increase SP expression, predominantly SP-D (14, 15). *In vitro*, recombinant IL-4-induced production of SP-D but not SP-A by rat type II alveolar epithelial cells (35). In our study, SP-D correlated with the number of eosinophils, but we did not find a correlation of SP-D with Th₂ cytokines.

Surfactant protein-C and SP-A were specifically altered after allergen challenge and unlike SP-B and SP-D, did not change after saline challenge. Importantly, the most pronounced change from baseline was seen for SP-C, and SP-C was most closely related to both, surfactant dysfunction and the degree of the allergic inflammation. Therefore, SP-C appears of notable importance in asthma. The potential role of SP-C in asthma can include protection from loss of biophysical surfactant function in terminal airways because SP-C maintains a stable association of surfactant with the interface (36) and it permits resistance to surfactant inhibition by invading plasma proteins (37). Potential immunological effects of SP-C that have been highlighted in studies by Augusto et al. (38) have to be defined in asthma.

In conclusion, this study demonstrates specific allergen-induced alterations of SP levels in BAL fluid of asthmatics compared with healthy controls that are closely related to surfactant dysfunction and the degree of the allergic inflammation. Surfactant protein-B, SP-C and SP-D levels increase 24 h after allergen challenge while SP-A levels decrease. These alterations need to be further studied to understand the time course and the underlying mechanisms. This is especially important when exploring therapeutic strategies using SPs in asthma with regard to timing and dosing regimen.

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