

Children with absent surfactant protein D in bronchoalveolar lavage have more frequently pneumonia

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Surfactant protein D (SP-D) is an important component of the pulmonary host defense system. We hypothesized that bronchoalveolar lavage (BAL) SP-D levels are lower in children presenting with recurrent bronchitis, providing evidence for a role of SP-D in human respiratory diseases. SP-D levels in BAL were measured in 45 children, who suffered from recurrent bronchitis for an average of 2–3 yr. Clinical outcome was assessed 2 yr after BAL. For comparison, BAL fluids from 15 control children without respiratory symptoms were evaluated. Among the 45 children with recurrent bronchitis, 12 had no SP-D in their BAL at the time of investigation. These SP-D-deficient patients had more frequently pneumonias and their long-term outcome was worse than that of the children with detectable SP-D. No genetic cause could be identified for the SP-D deficiency. Among the children with recurrent bronchitis and SP-D clearly detectable in BAL, those with the diagnosis of allergic asthma had threefold elevated levels compared with controls. In accordance with animal and *in vitro* data, elevated SP-D concentrations in BAL may represent an up-regulation due to allergic airway inflammation. In contrast, SP-D deficiency due to consumption or failure to up-regulate SP-D may be linked to pulmonary morbidity in children.

Surfactant protein D (SP-D) is produced in the lungs by alveolar type II cells and Clara cells as well as bronchial and tracheal gland cells and secreted into the airspaces where it forms a large, oligomeric complex. Outside the lungs, SP-D is present in epithelial cells of many human tissues, including the salivary, sweat, and lachrymal glands, pancreas and bile ducts, renal tubules as well as gastrointestinal and genital tract (1, 2).

Surfactant protein D and pulmonary surfactant protein A (SP-A) are members of the collectin family, which in humans also includes the mannan-binding lectin, CL-L1, and CL-P1 (3, 4). Because of their ability to directly interact with carbohydrates on the surface of invading microorganisms, the collectins are central components of innate immunity mechanisms (5, 6).

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Animal and *in vitro* data have demonstrated that pulmonary SP-D facilitates the clearance of inhaled microbial pathogens like *Pseudomonas aeruginosa* (7), *Streptococcus pneumoniae* (8), and respiratory syncytial virus (9), whereas others (*H. influenza*, group B *Streptococcus*) remain unaffected (10, 11). SP-D not only binds to and aggregates microorganisms (12), but also regulates the response of the pulmonary antigen-presenting macrophages and dendritic cells (6). The extent depends on the presence or absence of microorganisms and cellular debris (13). In the presence of allergens and plenty of SP-D, Th₂-type pro-inflammatory reactions are suppressed (14, 15) and Th₁ cytokines are induced (16).

Despite the large amount of data obtained from genetically engineered animals and *in vitro*

models, not much is known about the role SP-D may play in humans and especially in the human lung. Measurements of SP-D serum level may give indirect evidence about the changes possibly occurring within the lungs. The lung is the major synthesis site of SP-D and a spillover from this compartment into the systemic circulation may be the rate-limiting step (17–19). However, this concept is jeopardized by the recent observation of SP-D being present also in endothelial cells (20), suggesting another potential source for serum SP-D and complicating the interpretation of serum data.

In the lungs, SP-D concentrations in the airspaces are of special interest. Data on SP-D levels obtained by bronchoalveolar lavage (BAL) are, however, particularly rare for the more common and less severe conditions like asthma, pneumonia, and bronchitis (21). In 10 adult patients with asthma, Cheng et al. observed moderately increased levels of SP-D (148 % of control) (22), while Erpenbeck et al. found unchanged basal SP-D levels, which increased about twofold after segmental challenge (23).

Asthma and chronic bronchitis as well as community-acquired pneumonia constitute substantial health problems and a large socioeconomic burden, in particular in children (24). Asthma is the most common chronic illness among 4- to 12-yr olds. Chronic or chronic recurrent bronchitis, viral or transient wheeze, i.e. obstructive bronchitis, and atopic asthma are clinically very important diseases in preschool children (25).

The goal of the present study was to determine the SP-D concentrations in the BAL fluid of a group of children with long-standing recurrent bronchitis after the exclusion of other known diseases. We hypothesized that SP-D levels are lower in children with recurrent bronchitis, resulting in more frequent respiratory tract infections and associated chronic airway inflammation (26).

Subjects and methods

Study design

From a larger group of children with chronic and recurrent respiratory symptoms referred from other hospitals and physicians to the Department of Pediatric Pneumology at the Children's Hospital of the University of Munich for their assessment, BALs were performed in 76 consecutive children over a 2-yr period. Cystic fibrosis (sweat test, genetic testing when appropriate), primary ciliary dyskinesia (video microscopy of nasal or bronchial brushes, electron microscopy of the

cilia, measurement of nasal exhaled nitric oxide, scintigraphic transport studies if appropriate), bronchiectasis of other causes (chest computed tomography), and cellular or humoral immune deficiencies were excluded. In all children, chest radiographs, allergy and lung function tests (above the age of 5 yr) as well as an investigation for gastroesophageal reflux disease were carried out, if clinically indicated. The bronchoscopy with BAL was performed during a stable, relatively disease-free period and in the absence of an acute pulmonary exacerbation. Children with anatomic anomalies of the airways were excluded from the study. Lipid-laden macrophages and the differential cell count were assessed as a measure of the activity of airspace inflammation. Thirteen children were excluded because of insufficient amount of BAL fluid for investigations in addition to routine bacteriology and cytology. After extensive work-up, 45 children were left and diagnosed as having recurrent or chronic asthmatic bronchitis or non-obstructive bronchitis (Fig. 1).

Subjects

The clinical details of the patients included in the study are given in Table 1. The history of upper and lower respiratory tract symptoms, and the usage of medications were assessed at the time of bronchoscopy. Pneumonias were defined to be present when the characteristic clinical signs and symptoms (fever, tachypnoe, fine crackles, and reduced oxygen saturation) and a pathologic chest radiograph were present. The families were contacted by phone after an average time interval of 2.5 (2.3–2.8) yr (mean and 95% confidence interval) after the bronchoscopy and interviewed about the clinical outcome of their children in a standardized way.

The BAL fluids from the children without lung disease were aliquots obtained previously in a study that assessed inflammation in children with chronic tracheostoma (27). The lavages were performed during anesthesia for elective surgery for minor conditions. The study protocol was approved by the institutional review board, and written informed consent was obtained from the parents of the children and the older children before the study.

Bronchoalveolar lavage and sample preparation

Bronchoalveolar lavage was performed in the most affected lobe or in the right middle lobe using 4 × 1 ml of warmed 0.9% NaCl/kg body weight. The return from the first fraction was kept separate from the other three fractions,

Table 1. Clinical characteristics, treatment, and long-term outcome of the patients

	Recurrent bronchitis	Controls
Number of subjects (male)	45 (24)	15 (9)
Age at first symptoms (yr)	2.5 (1.4, 3.7)	NA
Age at BAL (yr)	4.8 (3.4, 6.2)	4.8 (2.7, 7.0)
History of recurrent upper respiratory tract infections, otitis, tonsillitis, acute laryngitis	31	5
History of asthma or history of bronchiolitis requiring hospitalization	24	0
Current treatment at BAL		
No medication	14	15
Inhaled β -agonists	23	0
Inhaled corticosteroids (cromolyns)	20 (7)	0 (0)
Antibiotics	6	0
Follow-up after BAL (yr)	2.5 (2.3–2.8)	NA
Outcome improved, loss of symptoms/deteriorated or same problems/unknown	31/6/8	NA

NA, not applicable; BAL, bronchoalveolar lavage. Data are numbers of subjects or means, lower 95% confidence interval of the mean, and upper 95% confidence interval of the mean.

which were pooled. Fractions 2–4 were filtered through sterile 160 μ m gauze. All manipulations were performed immediately on ice. Aliquots of the cell supernatant were stored at -80°C prior to analysis. Duration of storage was from 3 to 5 yr.

Biochemical methods

Antisera and standards. The antibodies against SP-D (P13, rabbit polyclonal anti-human SP-D) were a gift of Prof. Erica Crouch (Dept. of Pathology, St. Louis, MO, USA) and from BMA, Augst, Switzerland (monoclonal mouse anti rat SP-D, 100% crossreactive to human SP-D, but not to SP-A). As standards, human alveolar proteinosis SP-D was used which gave the same result as human recombinant SP-D. For better comparisons, the concentration of the standard was adjusted to that of a recent study using a commercial ELISA system (28), which yields values about eightfold higher than those given in our previous BAL studies.

Surfactant protein concentrations and characterization. The concentration of SP-D was assessed by ELISA, as described in detail elsewhere (27, 29). The lower limit of detection of the assay was about 15 ng/ml. The intra-assay coefficient of variation was 5.4%, the inter-assay coefficient of variation was 15.1%.

Total protein content of the samples was determined with the Bio-Rad Protein Assay Kit (Bio-Rad, Richmond, CA, USA), which is based on the method by Bradford (30). For one-

dimensional electrophoresis and Western blotting, 10–25 μ g of total protein was resuspended in LDS sample buffer and separated under reducing conditions on NuPage 10% Bis-Tris gels in MOPS-SDS running buffer, using a NOVEX X-cell II Mini-Cell system (Novex, San Diego, CA, USA). For immunodetection, the proteins were transferred onto a PVDF membrane (Immobilon P; Millipore, Bedford, MA, USA) with a NuPage Blot module (Novex) according to the manufacturer's recommendations. SP-D was detected by the polyclonal rabbit antisera and the enhanced chemiluminescence assay (Amersham Biosciences, Buckinghamshire, UK) with horseradish peroxidase-conjugated goat anti-rabbit polyclonal anti-IgG (1:10,000; Dianova, Hamburg, Germany), followed by exposure to X-ray film (Hyperfilm ECL; Amersham Biosciences). Under these conditions, the assay could detect about 2 ng of SP-D per lane, as determined in dilution experiments. Two hundred nanograms of SP-D was used as a standard (Fig. 2). As the signal from western blotting was only linear to SP-D concentrations in the lower range and reached a plateau at higher amounts, no quantitative SP-D values were derived from this technique.

Assessment of genetic variations regulating SP-D levels. It was not the primary goal of this study to investigate the genetic causes of the lack of SP-D in BAL. After we identified subjects with no SP-D in their BAL, common polymorphisms known to be associated with low serum SP-D levels were analyzed in those subjects, from whom informed consent could be obtained and DNA was available.

The M31T, A180T, and S290T substitutions encoded by exons 1, 4, and 7, respectively, of the *SFTPD* gene were analyzed. DNA was available from 10 out of the 12 children, who had no SP-D detectable in their BAL by ELISA ($n = 6$) and western blotting ($n = 4$). For comparison, eight children were selected with the same clinical characteristics, but detectable SP-D in their BAL.

To identify these polymorphisms, exon 1 (forward primer: 5'-tcttctctctctctgcactg-3', reverse primer: 5'-gaccaggcaggccactc-3'), exon 4 (forward: 5'-gacctgcaggccctaa-3', reverse: 5'-tgtaggcattgacagctccaa-3'), and exon 7 (forward: 5'-gtgcacacaggetgatgg-3', reverse: 5'-ccctctgtcttggatcagtcac-3') were amplified in a 25 μ l-reaction mixture containing 50 ng of template DNA, 2.5 μ l of 10 $\times$ PCR buffer without MgCl_2 , 2 μ l MgCl_2 (25 mM), 1.25 μ l dNTP mix (10 mM), 1 μ l of each primer (10 μ M), and 0.1 μ l Hot-

StarTaq (Qiagen, Hilden, Germany). The cycling conditions were as follows: initial denaturation at 95°C for 10 min, followed by 48 cycles at 95°C for 20 s, 58°C for 40 s and 72°C for 90 s, and a final extension at 72°C for 120 s. The PCR products were purified with the QIAquick PCR Purification kit (Qiagen) and sequenced with the ABI PRISM Big Dye Terminator v3.1 Ready Reaction Cycle Sequencing kit (Applied Biosystems, Darmstadt, Germany), using an ABI 3130 Genetic Analyzer.

Additionally, part of the promotor (nucleotides –600 bis + 79) was sequenced in 10 patients with the forward primer 5'-ttacaagaacagtgcacaagac-3' and the reverse primer 5'-gctgtagggtcattgtgcaac-3'. Amplification was performed as described above.

Statistical analyses

Data are presented as means and lower and upper 95% confidence intervals of the mean. Differences between two groups were calculated by the two-sided *t*-test. When three groups were compared, ANOVA with Tukey's multiple comparisons *post hoc* test was performed. Correlations were assessed using Pearson's correlation test. Frequencies were compared using the chi-squared test (Prism 4.00; GraphPad Software, San Diego, CA, USA). A *p*-value <0.05 was considered significant.

Results

The 45 children with recurrent bronchitis had a history of several years of recurrent upper and lower respiratory tract symptoms, before they were assessed in our clinic. Diseases such as cystic fibrosis, primary ciliary dyskinesia, immune deficiencies, and anatomic bronchial anomalies were excluded. The general clinical outcome 2.5 yr after the diagnostic BAL was good with improvement or complete recovery in the majority of cases (Table 1). Thus, these children represent the typical group of patients treated in pediatric pulmonology departments (Table 1). The BAL in these children showed increased numbers of neutrophils and lymphocytes at the expense of alveolar macrophages (Table 2). Potentially pathogenic bacteria were more frequently recovered than in the control group (Table 2).

All lavages were analyzed for SP-D by two independent methods. No SP-D was detected by ELISA in 12 children from the recurrent bronchitis group, but in none of the controls. The more sensitive and specific method of one-dimensional electrophoresis and western blotting demonstrated the complete absence of SP-D in

Table 2. Cellular and microbiological results of bronchoalveolar lavages

	Recurrent bronchitis	Controls	<i>p</i>
Recovery (%)	58.5 (53.3–63.8) 35	58.1 (50.3–65.9) 15	NS
Cell viability (%)	80.6 (76.4–84.8) 40	83.1 (75.8–90.3) 14	NS
Total cells (/μl)	207 (147–268) 45	179 (64–294) 14	NS
Macrophages (%)	74.4 (67.9–80.8) 42	88.4 (85.1–91.8) 14	0.0158
Lymphocytes (%)	13.7 (10.4–17.0) 42	2.0 (0.5–3.5) 14	0.0002
Neutrophils (%)	10.6 (5.4–15.7) 42	1.3 (0.6–2.0) 14	0.0407
Eosinophils (%)	1.6 (0.6–2.7) 42	1.3 (0.6–2.0) 14	NS
Mast cells (%)	0.0 (0.0–0.1) 42	0.0 (0.0–0.0) 14	NS
No bacteria	6/36*	13/15	<0.0001
Normal upper respiratory tract bacteria	22/36*	2/15	0.0047
Potentially pathogenic bacteria†	16/36*	0/15	0.0018

Data are presented as means, lower 95% confidence interval of the mean, and upper 95% confidence interval of the mean from *n* subjects. Differences were calculated by the two-sided *t*-test for the cellular components and by the chi-squared test for comparison of frequencies. NS, not significant.

*Different compared to controls.

†The potentially pathogenic bacteria recovered in BAL included *Haemophilus influenzae*, *Pneumococci*, *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella oxytoca* or *pneumoniae*, and *Moraxella catharralis*.

four of these children (Fig. 2) and low levels in the remaining eight children. The rest of the children had abundant SP-D that was detected in the 47–51 kDa range (Fig. 2). These two groups of children are presented separately (Fig. 1).

Children with no SP-D detectable by ELISA in their lavage

The 12 children with SP-D deficiency in BAL did not differ in age, sex, age at first symptoms, age at BAL, frequency of history of upper history tract infections, croup, bronchiolitis, and asthma. However, the number of pneumonias per patient was significantly higher in children with SP-D deficiency compared to those without. In addition, patients with more than four pneumonias in their life were more frequently found in the group with no SP-D detectable in their BAL (Table 3).

The children with SP-D deficiency in their BAL also had a worse long-term outcome than those without. Thirty-seven of the 45 children were re-evaluated after a mean of 2.5 yr. Out of the 26 children with SP-D present in their BAL by ELISA, 24 had recovered, whereas two patients showed worse or constant symptoms. In contrast, of the 11 children with SP-D deficiency in BAL retrieved for follow-up, four had the same or worse symptoms (*p* = 0.0306, chi-squared test, odds ratio 6.86 [95% confidence interval 1.03–45.6]).

Two approaches were made to determine the cause for the low or absent SP-D. First, the

Table 3. Frequency of pneumonias and presence or deficiency of surfactant protein D

	SP-D assessed by ELISA			SP-D assessed by western blotting		
	SP-D present	SP-D deficiency		SP-D present	SP-D deficiency	
Number of patients	32	12		40	4	
Number of pneumonias per patient	1.2 (37 in 32 patients)	4.1 (49 in 12 patients)	Frequency 3.5-fold higher	1.5 (62 in 40 patients)	6.3 (25 in four patients)	Frequency 4.5-fold higher
Patients with 0–3 pneumonias	31	8		37	2	
Patients with four or more pneumonias	1	4	$p = 0.0049$ OR 15.5 (1.5–160)	3	2	$p = 0.0107$ OR 12.3 (1.3–121)

Because, in one patient, the frequency of pneumonias could not be assessed with certainty, only 44 patients were included. Differences in frequency were calculated using the chi-squared test. Odds ratio (OR) and 95% confidence interval are given.

frequency of amino acid substitutions known to be associated with lower serum SP-D levels (31, 32) was investigated. No differences were found between the cases and the comparison group ($p = 1$) (Table 4). Therefore, in a second approach, the promotor region, which is known to regulate SP-D transcription, was sequenced. Only one heterozygous mutation was detected at nucleic acid position –477, not being located in a known C/EBP (transcription factor) binding region (Table 4). Thus, none of the known genetic causes for low SP-D levels was identified in these subjects.

Children with SP-D detectable in BAL and recurrent bronchitis

The children with detectable SP-D in their BAL and recurrent bronchitis had elevated concentrations of SP-D in their lavages (Table 5). The amount of SP-D expressed per gram of protein was also increased (Table 5). The increased SP-D concentration in the group of children with recurrent bronchitis was primarily due to the children with asthma, whereas, in those with non-obstructive bronchitis, the levels of SP-D were not significantly elevated (Fig. 3). No correlations between the number of neutrophils or other cellular elements in BAL were observed. There was also no relationship to the presence or absence of bacteria recovered in BAL fluid.

Discussion

In our cohort of 45 children with recurrent bronchitis, 12 had no SP-D in their BAL detectable by ELISA. These SP-D-deficient patients experienced more frequently pneumonias, and their clinical outcome on follow-up was worse than that of the children with detectable SP-D. Among the children with recurrent bronchitis and SP-D clearly detectable in BAL, those with the diagnosis of allergic asthma had three-fold elevated levels of SP-D compared to the normal controls.

Recurrent bronchitis and no SP-D in BAL

Initially, we had hypothesized that SP-D levels are lower in children with recurrent bronchitis, but we were surprised to find a high number of children with no SP-D in this cohort of subjects. These children clearly differed from the other children with respect to the frequency of pneumonias and clinical outcome, supporting the hypothesis derived from animal experiments that low SP-D levels are associated with lower respiratory tract infections and with airway inflammation.

Although our finding of a local SP-D deficiency is very plausible, it raises some questions. We do not know whether the lack of SP-D is persistent or temporary, i.e., hereditary or regu-

Table 4. Analysis of the *SFTPD* gene with respect to polymorphisms known to be associated with low SP-D serum levels (M31T, A180T, S290T) and abnormalities in the regulatory promotor region

Allele	M31T			A180T			S290T			Promotor (–600 to +79)
	M/M	M/T	T/T	A/A	A/T	T/T	S/S	S/T	T/T	
Cases with SP-D deficiency (n = 7)*	1	4	2	3	3	1	2	5	0	No mutation in 9 of 10 DNA samples; heterozygous C > A mutation at nucleic acid position –417 in 1 of 10 samples Not analyzed
Controls (n = 8)	1	5	2	2	3	3	2	6	0	

SP-D, surfactant protein D.

*PCR reaction was successful in 7 of 10 DNA samples available.

Table 5. Concentrations of surfactant protein D (SP-D) and total protein in bronchoalveolar lavages

	Recurrent bronchitis	Controls	p
SP-D (ng/ml)	447.2 (350.4–544.0) 33	176.0 (104.8–247.2) 15	0.0007
Total protein (μg/ml)	102.3 (81.8–122.7) 33	74.9 (66.0–83.9) 15	NS
SP-D/protein (%)	0.56 (0.40–0.80) 33	0.24 (0.16–0.32) 15	0.0143

Data are presented as means, lower 95% confidence interval of the mean, and upper 95% confidence interval of the mean from n subjects. Differences were calculated by the two-sided *t*-test. SP-D levels were determined by ELISA. The patients with SP-D deficiency, i.e., a level below the lower limit of detection, are not included in the table. NS, not significant.

latory. To answer the latter question, repetitive analysis of BAL is definitely necessary. This is unfeasible in our patients, because they were bronchoscoped and lavaged to exclude anatomic abnormalities and to assess airspace inflammation during a unique diagnostic work-up. Not much is known about changes in SP-D in the bronchoalveolar space with time. In subjects with cystic fibrosis and mild lung disease, i.e., FEV₁ above 80% of predicted value, sequential analysis repeated thrice over a 3-yr period showed very stable SP-D concentrations in the lungs (33). Of the 20 patients assessed in that study, only one patient, who had initially SP-D concentrations in the lower range, was found to have a single value out of 60 lavages which was zero (33). Furthermore, these cystic fibrosis patients with mild lung disease had a more severe endobronchial inflammation, i.e., between 18% and 40% of neutrophils in BAL than observed in the present study.

As SP-D levels normally decrease due to a more severe inflammation (27, 34, 35), our data argue against a down-regulation of SP-D synthesis as the sole cause based on the degree of neutrophilic or eosinophilic airway inflammation observed in the patients investigated here.

Proteolytic degradation has been described to also play an important role (36, 37). However, in this study, all samples were analyzed using sensitive western blots, and no proteolytic SP-D degradation products were detected in any of these patients. Other mechanisms, e.g., genetic or regulatory, may therefore be involved.

Recently the Thr/Thr/Thr phenotype at positions 31, 180, and 290 of SP-D was shown to be associated with lower serum SP-D levels (31, 32). We investigated whether the respective nucleotide substitutions were more frequently present in children with no SP-D in their BAL. However, among the cases and controls, who gave their consent for genetic analysis and from whom DNA was available, the same allele frequency was found.

Transcriptional regulation, e.g., through AP-1, HNF-3, aGT box, or C/EBF elements in the promotor region of the *SFTPD* gene, may also be involved in the regulation of SP-D concentrations in the lungs (38). Therefore, we additionally searched for mutations in this gene region. Except for one patient, no base changes were found, excluding mutations in the transcription factor binding sites analyzed as causes for the low SP-D levels observed.

Taken together, our data show that children with recurrent bronchitis and no detectable SP-D in their BAL experience more frequently pneumonias and have a worse long-term outcome than those with SP-D. This observation needs to be confirmed prospectively in epidemiologic studies. Short-term kinetics of SP-D levels in the lungs are necessary to unequivocally determine the causes of the observed SP-D deficiency. Such studies may be performed in older children or in adults using induced sputum, although major methodological difficulties have still to be overcome for such an analysis (39).

Children with SP-D detectable in BAL and recurrent bronchitis

The children with recurrent bronchitis represent a characteristic group of children typically referred to the pediatric pulmonologist for diagnostic and therapeutic work-up. A precise phenotypic characterization of these children is rather difficult. Attempts to classify such children have focused on the presumed etiology, e.g., episodic viral cough or wheeze, persistent cough or wheeze induced by multiple triggers, or wheeze resulting from developmental differences in lung mechanics (40). Another approach focuses on the time course of the disease, e.g., transient, persistent, or late-onset (41). We used two categories instead – children with asthma or children with bronchitis (Fig. 1). Children with asthma were defined as having a personal and/or family history of atopy, allergic sensitizations, and recurrent episodes of wheeze. Children with bronchitis were characterized by recurrent non-wheezy episodes of cough, a lack of a history of atopy, and no allergic sensitizations.

The children with asthma had an about threefold increase in SP-D in their lavage compared to the controls (Fig. 3). This extends observations made in adult patients with asthma into early childhood (22). Thus, children with asthma and of very young age likely also have an up-regulated bronchoalveolar level of SP-D. The amino acid variants of SP-D known to be associated with altered levels of SP-D were recently shown not to play a major role in the

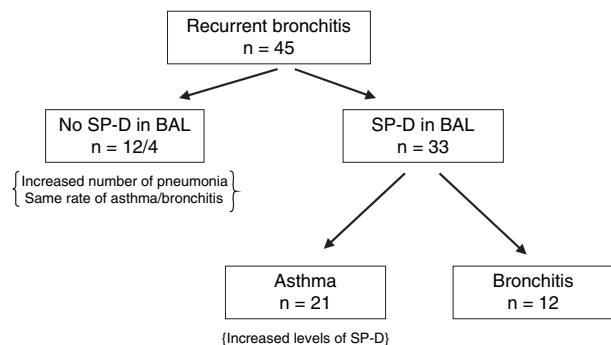


Fig. 1. Overview of study subjects. No surfactant protein D (SP-D) was found in bronchoalveolar lavage (BAL) by ELISA in 12 children and, with the more sensitive western blotting method, in four children.

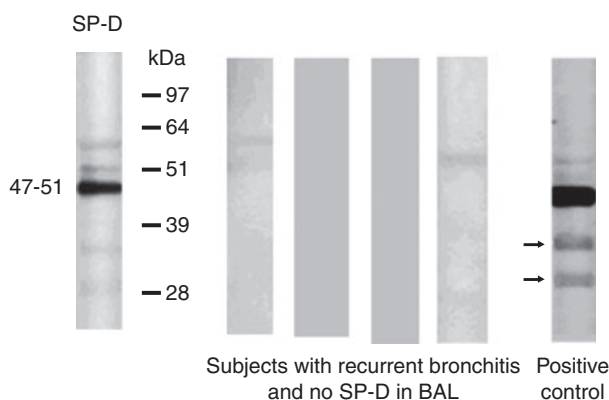


Fig. 2. Western blotting of bronchoalveolar lavages. Two hundred nanograms of human recombinant surfactant protein D (SP-D) standard with the characteristic molecular weight was easily detectable (left lane). No SP-D (< 2 ng/ml) was detected in one patient with recurrent bronchitis (second gel from left) and in three patients with asthma. Gels were run at three different time points with a load of up to $25 \mu\text{g}$ of total protein per lane under reducing conditions. The four probands with complete SP-D deficiency and also the other eight patients, who had no SP-D detectable by ELISA assay, had a significantly increased frequency of pneumonias (see Table 3). On the right-hand side, a sample from a patient with chronic bronchitis and cystic fibrosis was added as a positive control. It clearly shows SP-D degradation products, as indicated by the two arrows.

genetic predisposition to bronchial asthma in children (42).

Numerous studies have shown that adults and children with an allergic asthma have a Th2 lymphocyte inflammation dominated by a high expression of interleukin (IL)-4 and IL-13 (14). Interestingly, mice overexpressing IL-4 (43) and IL-13 (44) all show markedly elevated SP-D expression in their lungs. Additionally, IL-4 has been shown to directly and specifically enhance SP-D expression in type II alveolar epithelial cells (45). Therefore, it is likely that in the

Bronchoalveolar SP-D deficiency

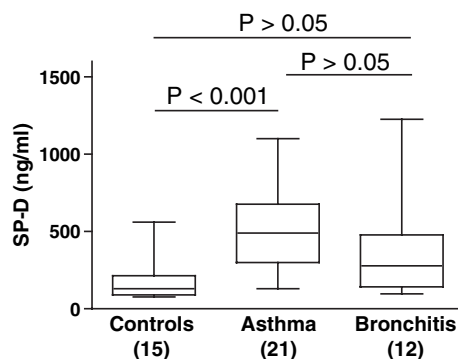


Fig. 3. Surfactant protein D (SP-D) levels in the bronchoalveolar lavage as determined by ELISA were significantly elevated in children with asthma compared to controls. The number of patients analyzed is given in brackets below the legend labels. Data are presented as box and whisker plots, i.e., the box represents the interquartile range which contains the 50% values. The whiskers are lines that extend from the box to the highest and lowest values. The line across the box indicates the median. Differences were calculated using ANOVA with Tukey's multiple comparisons *post hoc* test.

asthmatic children investigated here, the increased SP-D levels were related to their allergic airway inflammation. As recently reviewed by Haczku et al. (14), the functional role for elevated SP-D level is a negative feedback regulation of the allergic inflammatory changes in the lung, as the Th2-type inflammation is strongly inhibited by SP-D. Taken together, SP-D is elevated in asthmatic children, which may be the result of a Th2-dominated airway inflammation.

Although this study has several strengths, including the independent assessment of SP-D by two different methods, i.e., ELISA and western blotting, and the investigation of a large population of children with bronchoscopic BAL, some limitations must be considered when interpreting the results. The study was performed in a single center, the setup to obtain the information regarding the frequency of pneumonias was retrospective, and, finally, neither bronchial biopsies nor comprehensive genetic studies were performed to investigate the causes of the changes in the SP-D level.

In summary, after exclusion of known causes such as anatomic abnormalities, cystic fibrosis, primary ciliary dyskinesia, or immune deficiencies, some children with recurrent bronchitis have no SP-D in their BAL. These individuals are characterized by an increased frequency of pneumonias and a worse clinical outcome during follow-up. In contrast, children with allergic asthma have elevated levels of SP-D compared to healthy controls.

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