

Pulmonary surfactant in cystic fibrosis

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ABSTRACT: Cystic fibrosis (CF) leads to a chronic inflammation of the airways with significant air flow limitations developing early in the course of the disease. As a well-functioning pulmonary surfactant is necessary to keep the alveoli and the small conducting airways open during expiration, we hypothesized that the biochemical and biophysical properties of surfactant may be impaired in CF.

Bronchoalveolar lavage fluid obtained during a clinically stable period was analysed from 20 CF patients (5.9–20 yrs) and 17 healthy children and adults.

CF patients had significantly elevated total and polymorphonuclear neutrophil cell counts, whereas the concentrations of total protein and phospholipids did not differ from controls. The percentage of surface active phospholipids, phosphatidylcholine and phosphatidylglycerol, and the concentration of surfactant protein A were significantly reduced in CF patients. Surfactant protein B was unchanged. Although the relative proportion of large aggregates was higher in CF, their surface active properties were inferior, as assessed in the pulsating bubble surfactometer. Because the capacity of CF lavage fractions to inhibit surfactant function was the same as that of controls, impaired minimal surface tension was more likely to be due to the biochemical alterations detected, than to inhibition of a well-functioning surfactant.

The impaired pulmonary surfactant system in clinically stable patients with cystic fibrosis is in agreement with the view that surfactant dysfunction may contribute to lung disease in cystic fibrosis.

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Cystic fibrosis (CF) is a lethal recessive genetic disorder caused by mutations in the cystic fibrosis transmembrane conductance regulator, which results in pathological airway secretions and a chronic inflammatory condition of the airways. In CF lung disease the small airways are affected at an early stage, resulting in obstructive air flow limitations with consequent overinflation, or in areas of collapsed lung tissue [1].

The presence of sufficient, well-functioning pulmonary surfactant is necessary to prevent the alveoli from collapsing at end-expiration. Increasing evidence suggests that surfactant is needed not only in the most terminal parts of the airways, but also in the narrow section of the bronchi through which air is conducted to the alveoli [2–4]. *In vitro* and *in vivo* studies showed that a lack of surfactant led to the closure of the small cylindrical airways. Furthermore, the presence of phospholipases, proteases and transudated plasma proteins in the airways during inflammatory reactions, might severely disrupt the functional ability of surfactant to keep the conducting airways open [5, 6]. There is not much information available on the biophysical surface activity and biochemical composition of surfactant in CF patients. In comparison to asthmatic patients, bronchoalveolar lavage (BAL) fluid in CF revealed an elevated level of phosphatidylethanolamine and phosphatidylglycerol, whereas the degree of saturation of the major surface active phospholipid, phosphatidylcholine, was reduced

[7]. Reports comparing CF patients with normal patient groups are not available. However, preliminary data suggest a decreased content of SP-A [8, 9]. In lavages of the main bronchi, an extremely depressed fraction of phosphatidylcholine (3% of phospholipids) compared to 55% in controls, and an increased surface tension, was noticed [10].

Insufficient or biophysically impaired surfactant may play a significant role in the sequence of pathophysiological events that results in reduced function in CF patients. A detailed knowledge of the biophysical and biochemical characteristics of surfactant in CF is therefore essential. In this study we sought to obtain these data from young, stable patients with CF, to compare them to healthy subjects and to identify deficiencies which could be specifically targeted in future treatment. Some of the data have already been presented in abstract form [11].

Materials and methods

Control subjects

We studied 14 healthy children and young adults (age range 4–20 yrs) and three older adults (50, 51 and 63 yrs), all nonsmokers (table 1). None of the subjects had a history of chronic respiratory symptoms, upper or lower

Table 1. — Age, clinical condition and cellular content of bronchoalveolar lavage (BAL)

| | Controls | CF | p-value |
|---|--------------------|----------------------|---------|
| Age yrs | 17.5±4.3 (4–51) | 15.1±1.6 (5.9–24) | NS |
| Berner score | 25 | 21.6±0.5 (16–24) | <0.001 |
| Sputum positive for: | | | |
| <i>Pseudomonas aeruginosa</i> n | 0 | 12 | |
| <i>Aspergillus spp.</i> n | 0 | 5 | |
| Total cell count ×10 ⁴ cells·mL ⁻¹ | 15±1.8 (11–31) | 106±21 (21–340) | <0.05 |
| Macrophages % | 89.6±1.5 | 42.7±6.0 | <0.01 |
| Lymphocytes % | 8.6±1.4 | 7.9±1.0 | NS |
| Neutrophils % | 1.8±0.4 | 48±6.5 | <0.001 |
| Eosinophils % | 0.2±0.1 | 0.3±0.1 | NS |

Differences between control and cystic fibrosis (CF) were evaluated by t-test. Data are presented as mean±SEM, and range in parenthesis, or as absolute number (n), from 17 control patients and 20 CF patients. NS: nonsignificant.

respiratory tract infection in the preceding 2 months or a history of a hyperreactive bronchial system, while all had normal forced expiratory volume in one second (FEV₁) values. Lung function tests were not performed in two children who were too young to comply with the procedure. All children and young adults were undergoing elective surgery for nonpulmonary diseases, like herniotomies, orchidopexias, correction of urogenital anomalies and removal of cutaneous nevi. After informed consent, BAL was performed under general anaesthesia (N₂O, O₂, 1–5% halothane) and tracheal intubation with a paediatric (3.5 mm) or adult (4.9 mm) fiberoptic bronchoscope before surgery. The three older adults were lavaged to exclude pulmonary pathology potentially associated with earlier job-related exposure to asbestos fibres. All of these three subjects had normal cell numbers, differential cell counts and, biochemical parameters in their lavages, obtained by flexible bronchoscopy under local anaesthesia, and were included in the control group.

CF patients

The 20 patients with CF (age range 5.9–20 yrs) were randomly selected from the population of those attending the out-patient department of the CF clinics. The diagnosis was established by positive sweat tests, and by the characteristic clinical course. At the time of the evaluation, none of the subjects had an acute exacerbation of the lung disease, and there had been no changes in therapy in the preceding 4 weeks. Clinical condition was quantitated with the Berner CF score [12] (table 1). This scoring system is used in Switzerland and Germany. It evaluates the overall clinical status, the antibiotic therapies necessary, the pulmonary condition, the severity of gastroenterological involvement and the Crispin-Norman score. A maximum of 25 points was divided among five clinical states of severity. A Berner score of 25–21 or 20–16 is equivalent to about 100–80 or 80–60 points in the Shwachman scoring system. There is a close linear relationship between these two scoring systems. Lung function testing was performed in all but two very young patients with CF. FEV₁ was

62.2±3.1% of predicted and forced vital capacity (FVC) was 72.1±2.1% pred. BAL was performed with a flexible bronchoscope, under local anaesthesia. The study protocol was approved by the institutional review boards and informed consent was obtained from the parents, older children and adults before the study.

BAL procedure

For BAL a flexible paediatric 3.5 or an adult 4.9 mm bronchoscope was wedged in the right middle lobe or in one of its segments. Saline (0.9%), warmed to body temperature, was used. Three times the amount of 1 mL·kg body weight⁻¹ was instilled and immediately withdrawn by gentle suction. The recovery of BAL fluid did not differ between the groups and was on average 57±12% of instilled volume. All three fractions were pooled, an aliquot was used to obtain the total cell count in haemocytometer and cytospin preparations were stained according to May-Grünwald-Giemsa. After filtration over gaze and centrifugation at 200×g for 10 min, the supernatant was stored at -70°C.

BAL fractions

BAL supernatant was either used directly, or, fractions with different surfactant subtypes were prepared by centrifugation. A surface active fraction containing ultraheavy and heavy forms, called the large surfactant aggregates (LA), can be separated by differential centrifugation at 40,000×g and 4°C for 30 min from the small aggregates (SA), which have lighter forms and less surface activity [13–15].

Phospholipid analysis

As described before [15], aliquots of the samples were lipid extracted, the lower phase was Folch washed and solvents were removed under a stream of N₂. Phospholipid content was determined from the inorganic phosphate content of the lipid extracts. Phospholipids were separated by high performance thin layer chromatography (HPTLC) in a solvent system containing chloroform, methanol, petroleum ether, acetic acid and boric acid (40:20:30:10:1.8 v/v/v/v/w). Phospholipid distribution was assessed from the phosphorus content of the individual spots, which were identified by authentic standards. All measurements were performed in duplicate.

Enzyme-linked immunosorbent assay (ELISA) for surfactant protein A (SP-A)

96-well plates (Maxisorp, Nunc, Wiesbaden, Germany) were coated with the anti-SP-A monoclonal antibody PC-6 (a gift from R. Hasegawa, Teijin, Yamaguchi, Japan) in carbonate buffer (Na₂CO₃ 0.015 M, NaHCO₃ 0.035 M, pH 9.6). After blockade (phosphate-buffered saline (PBS)) with 0.1% Tween 20, 1% bovine serum albumin (BSA), pH 7.4) the samples were added and the plate incubated, and washed twice. The bound SP-A was then detected with a rabbit polyclonal antihuman SP-A antibody (gift of W. Steinhilber, Byk-Gulden, Konstanz, Germany). After two washes, goat anti-rabbit-immunoglobulin G (IgG), coupled to peroxidase (Sigma, Deisenhofen, Germany)

was added. The plate was subsequently washed three times, and ABTS-substrate (Boehringer-Mannheim, Mannheim, Germany) and H₂O₂ were used for colour development. The plate was read at 405 nm. Human recombinant SP-A (0–400 ng·mL⁻¹; a gift of W. Steinhilber, Byk-Gulden, Konstanz, Germany) was used as the standard. The lower limit of detection was approximately 25 ng·mL⁻¹. All samples were assessed in duplicate and serially diluted to select the appropriate linear range of the assay.

ELISA for surfactant protein B (SP-B)

SP-B was determined as described by KRÄMER *et al.* [16]. Briefly, samples were mixed with propanol (1:1 v/v) to achieve a homogenous dispersion for adsorption onto the wells of a microtitre plate. After drying, trifluoroethanol was added to enhance the binding of SP-B to the polystyrol plate and the phospholipids were selectively removed by washing procedures with diisopropylether/butanol and Tween 20. Solid phase bound SP-B was detected by a monoclonal mouse antibody directed against SP-B (gift of Y. Suzuki, Kyoto, Japan). In order to detect binding, a biotinylated antimouse antibody and avidin coupled peroxidase was used. The assay allowed the reproducible determination of SP-B in a range of 0.3–40 ng SP-B·well⁻¹. Human SP-B dimer (in 1-propanol/PBS (1:1); 4 mg·mL⁻¹) was used as the standard, and was a gift of W. Seeger, Gießen, Germany. All samples were serially diluted and the appropriate linear range was selected for determination of concentration. The samples were assessed in duplicate.

Surface tension

The surface tension modifying properties of the specimens were evaluated in a pulsating bubble surfactometer (Electronetics, Amerherst, NY, USA). Before measurement, the samples were resuspended at a phospholipid concentration of 3 mg·mL⁻¹ in 0.9% saline with 3 mM CaCl₂, 37°C. Adsorption (γ_{ads}) was defined as the surface tension obtained 10 s after formation of the bubble. Minimum surface tension (γ_{min}) was the surface tension in the equilibrium after 3 min of pulsations (20 cycles·min⁻¹; minimum (0.40 mm) and maximum (0.55 mm radius)), read at minimum radius of the bubble by an automated computer program (fastbubs by D. Bejarneson, University of Western Ontario, Canada).

Inhibition of surfactant function

To determine the inhibitory potential of soluble BAL constituents, the supernatant of the 40,000×g fraction (the small aggregates (SA) fraction) was tested for its effectiveness in inhibiting a functionally active surfactant. After the determination of total protein content (Biorad, Munich, Germany) and lypophilization in a centrifuge under reduced pressure (Vacuum concentrator, Bachhofen, Munich, Germany), the samples

were reconstituted in water in such a way that the final protein concentration was 4 g·L⁻¹ after being mixed with the natural bovine surfactant (phospholipids 0.5 g·L⁻¹). For purposes of comparison, the surfactant was analysed in the absence of protein and with serum at various concentrations ranging 0.5–4 g·L⁻¹. The mixture was vortexed and measured in the bubble surfactometer as described above.

Surface area cycling

The conversion of large aggregates (LA) surfactant fraction into the SA fraction can be studied by an *in vitro* system, the surface area cycling [13, 14]. LA obtained from the cell-free BAL by centrifugation at 40,000×g for 30 min were resuspended in conversion buffer (NaCl 150 mM, Tris 10 mM, CaCl₂ 1 mM, MgCl₂ 1 mM, ethylene diaminetetra-acetic acid (EDTA) 0.1 mM, pH 7.4) at a concentration of approximately 0.1 mg·mL⁻¹. Two millilitres of each sample was placed in plastic tubes (Falcon 2058, Becton Dickinson, NJ, USA) capped and attached to a rotator. The tubes were cycled at 40 rpm at 37°C for 3 h, so that the surface area changed from 1.1 to 9.0 cm² twice each cycle, exactly as described previously [13]. Control experiments were performed in parallel with other aliquots of the samples under identical conditions, but without cycling. At the end of the incubation period, SA and LA were separated by centrifugation as described above.

Statistical analysis

All data are presented as mean±standard error of the mean (SEM). Correlation analysis was performed by calculating the two-tailed Spearman correlation coefficient. Comparisons were made by the two-sided t-test. A p-value of less than 0.05 was considered significant.

Results

In patients with CF, the total cell counts of BAL and the percentage of polymorphonuclear neutrophil granulocytes were significantly elevated, indicating the presence of an inflammatory reaction, despite the patients being clinically well and having relatively high Berner scores (table 1). The cell-free BAL supernatant did not differ in its content of total protein and phospholipids. The mass of SP-A, but not of SP-B, was, however, reduced (table 2). The phospholipid profile of CF patients was significantly altered, as the principal surface active lipid components of surfactant, phosphatidylcholine and

Table 2. — Content of protein, phospholipids, surfactant protein A (SP-A) and surfactant protein B (SP-B) in bronchoalveolar lavage (BAL)

| | Controls | | | CF | | | p-value |
|--|------------|-----------|----|------------|-----------|----|---------|
| | Mean±SEM | (range) | n | Mean±SEM | (range) | n | |
| Protein $\mu\text{g}\cdot\text{mL BAL}^{-1}$ | 204.0±77.3 | (26–974) | 15 | 268±59.0 | (92–720) | 12 | NS |
| PL $\mu\text{g}\cdot\text{mL BAL}^{-1}$ | 79.5±32.7 | (8–432) | 14 | 111.8±24.5 | (44–360) | 12 | NS |
| Protein/PL ratio | 4.4±1.1 | (0.6–16) | 14 | 2.7±0.48 | (0.8–6.7) | 12 | NS |
| SP-A $\mu\text{g}\cdot\text{mL BAL}^{-1}$ | 5.4±0.95 | (0.9–13) | 15 | 1.9±0.45 | (0.1–4.6) | 12 | <0.01 |
| % of PL | 19.8±4.23 | (1.6–44) | 14 | 1.9±0.37 | (0.1–3.4) | 12 | <0.01 |
| SP-B $\mu\text{g}\cdot\text{mL BAL}^{-1}$ | 2.9±0.63 | (0.6–6.0) | 13 | 4.0±0.75 | (1.1–7.7) | 12 | NS |
| % of PL | 6.9±1.30 | (1.0–17) | 13 | 2.5±0.53 | (1.6–6.8) | 12 | NS |

Differences between control and cystic fibrosis (CF) were evaluated by t-test. NS: nonsignificant; PL: phospholipid.

Table 3. – Phospholipid composition of the surfactant fraction of bronchoalveolar lavage from healthy controls and from patients with cystic fibrosis (CF)

| Phospholipid class | Controls | | CF | | p-value |
|----------------------------|----------|-------------|----------|--------------|---------|
| Lysophosphatidylcholine % | 0.7±0.4 | (0–2.1) | 2.6±0.8 | (0–9.7) | NS |
| Sphingomyelin % | 0.9±0.4 | (0–1.7) | 3.9±0.7 | (0.8–10.5)* | 0.019 |
| Phosphatidylcholine % | 73.4±3.6 | (72.2–79.6) | 62.3±2.7 | (36.9–76.2)* | 0.035 |
| Phosphatidylinositol % | 5.0±0.6 | (4.4–7.4) | 9.0±0.8 | (3.1–14.7)* | 0.015 |
| Phosphatidylserine % | 1.5±0.9 | (0–4.9) | 3.2±0.9 | (0–13.3) | NS |
| Phosphatidylethanolamine % | 4.4±2.0 | (1.2–12.3) | 8.2±1.6 | (0–21.9) | NS |
| Phosphatidylglycerol % | 9.7±2.1 | (7.8–13.1) | 6.2±0.8 | (0–10.6)* | 0.042 |
| Phosphatidic acid % | 2.4±0.6 | (1.6–4.6) | 1.9±0.4 | (0–3.4) | NS |
| Cardiolipin % | 0.7±0.5 | (0–2.6) | 0.8±0.2 | (0–2.3) | NS |

Values are the percentages of total phospholipid, presented as mean±SEM, and range in parenthesis, from five control subjects and 20 patients with CF. The p-values are derived from unpaired two-sided t-test.

Table 4. – Effect of surface area cycling on surfactant subfractions

| | Controls | CF | n |
|---|-----------|----------|------|
| No cycling | | | |
| LA | 92.4±3.3 | 92.0±0.9 | 11 |
| SA | 7.6±3.3 | 8.0±0.9 | 11 |
| Cycling | | | |
| LA | 48.0±3.4 | 56.2±3.8 | 11 |
| SA | 52.0±3.4 | 43.8±3.8 | 11 |
| Cycling with PMSF | | | |
| LA | 40.0±6.6 | 49.0±3.5 | 8 |
| SA | 60.0±6.6 | 51.0±3.5 | 7 |
| Cycling with α_1 -PI | | | |
| LA | 59.9±10.8 | - | 6 |
| SA | 40.1±10.8 | - | 6 |
| Final PL concentration $\mu\text{g}\cdot\text{mL}^{-1}$ | 136±15 | 96±5 | 6–11 |

The large aggregate fraction (LA) of surfactant was isolated and subjected to surface area cycling under different conditions, or to control incubation. After 3 h at 37°C, LAs, were separated from the small aggregates (SAs) by centrifugation at 40,000 g. α_1 -PI: α_1 -protease inhibitor (20 μM); PMSF: phenylmethylsulphonyl fluoride (5 mM); PL: phospholipid. No differences were detected by t-test.

phosphatidylglycerol were decreased (table 3). In CF patients, the ratio of SA to LA was significantly reduced (CF 0.25 ± 0.05 (n=17) versus controls 0.48 ± 0.09 (n=12); $p<0.05$).

Surface area cycling has been shown *in vitro*, in mice and dogs, to quantify the activity of a putative protease ("convertase") which may be involved in the alveolar surfactant metabolism of LA into SA [13, 14]. A substantial conversion, over a 3 h period, of the LA prepared from these lavages was, in fact, observed, although no differences were detected between

the control and CF patients (table 4). The presence of the protease inhibitors phenylmethylsulphonyl fluoride (PMSF) or α_1 -protease inhibitor had no significant effect on the LA conversion rate. These data suggest no impairment of convertase activity in CF samples when compared to controls.

The biophysical properties of the LA surfactant fraction were assessed in a pulsating bubble surfactometer. In the control subjects, the surface activity of the native surfactant was better than that from CF patients, at both the concentrations investigated (fig. 1). After lipid extraction, surface activity was generally reduced, indicating the presence of water soluble surface active components such as SP-A (fig. 1). The SA fraction is much less surface active and usually contains the majority of the nonsurfactant-associated proteins [17], such as serum proteins or other water soluble inhibitors of surfactant function [18]. To determine whether a strong inhibitory component might contribute to a reduced surface activity in these CF patients, the effect of the SA-fraction at a protein concentration of 4 $\text{g}\cdot\text{L}^{-1}$ was tested against a natural, bovine surfactant at a concentration of 0.5 $\text{g}\cdot\text{L}^{-1}$. Only a very slight impairment of γ_{min} was found. Apart from somewhat higher initial values (γ_{ads})

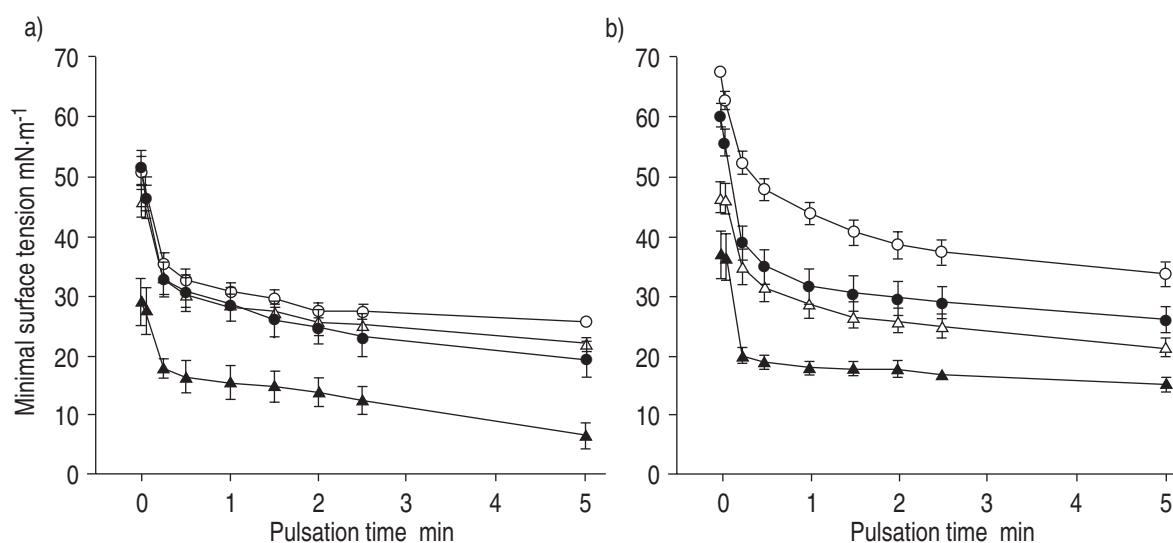


Fig. 1. – Minimal surface tension of the large aggregate fraction of the bronchoalveolar lavage from 8–11 healthy subjects (controls) and seven patients with cystic fibrosis (CF). Measurements were performed at phospholipid concentrations of: a) 3 $\text{mg}\cdot\text{mL}^{-1}$; and b) 1 $\text{mg}\cdot\text{mL}^{-1}$. ●, ▲: native surfactant fractions; ○, △: lipid-extracted fractions; ○, ●: CF patients; △, ▲: controls. Minimal surface tension in CF patients was significantly elevated at all time points ($p<0.05$) when compared to the control patients.

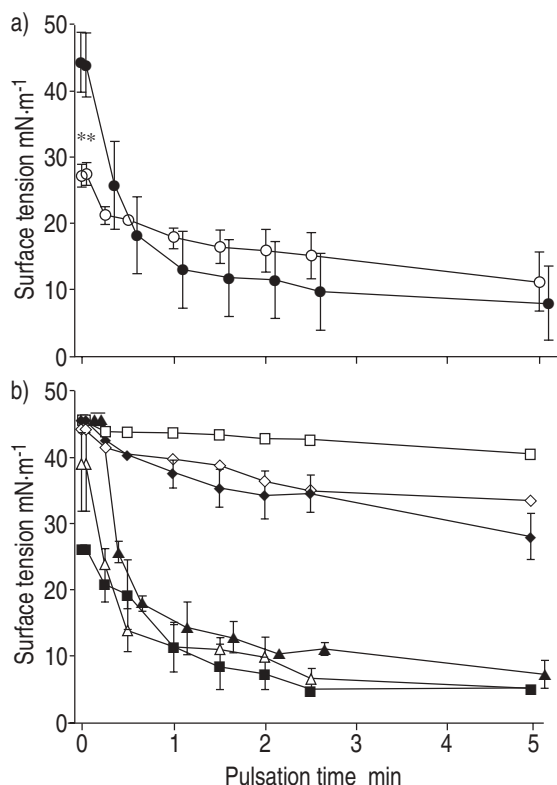


Fig. 2. – The inhibition of surfactant function by bronchoalveolar lavage (BAL) constituents and serum is shown. A natural bovine surfactant ($0.5 \text{ g phospholipid}\cdot\text{L}^{-1}$) was mixed with the proteinous small aggregate surfactant fraction isolated from BAL at a protein concentration of $4 \text{ g}\cdot\text{L}^{-1}$: a) no significant difference was observed between material from control ($n=6$) or cystic fibrosis (CF) patients ($n=6$) except for absorption (γ_{ads}) (**: $p<0.01$); b) for comparison the natural bovine surfactant was mixed with increasing concentrations of human serum ($n=2-4$). In the absence of additional protein, the surfactant effectively lowered the surface tension to very low values. \circ : controls; \bullet : CF; \blacksquare : surfactant only ($0.5 \text{ g}\cdot\text{L}^{-1}$); \triangle : surfactant $+0.5 \text{ g serum}\cdot\text{L}^{-1}$; \blacktriangle : surfactant $+0.75 \text{ g serum}\cdot\text{L}^{-1}$; \blacklozenge : surfactant $+1.0 \text{ g serum}\cdot\text{L}^{-1}$; \diamond : surfactant $+2.0 \text{ g serum}\cdot\text{L}^{-1}$; \square : surfactant $+4.0 \text{ g serum}\cdot\text{L}^{-1}$.

in CF patients, no other differences between control and CF subjects were detected (fig. 2a). For comparison, serum had a strong, dose-dependent, inhibitory effect that was maximal at $4 \text{ g}\cdot\text{L}^{-1}$, when assessed in the same system (fig. 2b).

Discussion

We observed less surface activity, an abnormal phospholipid composition, less SP-A and an unchanged SP-B content in surfactant from BAL fluid of patients with CF compared to healthy subjects. While these data clearly show the biophysical and biochemical impairment of surfactant, the exact mechanisms responsible for this are less apparent.

The biochemical changes of surfactant in these stable CF patients are sufficient to account for the reduced surface activity. Very similar changes, such as shifts in the composition of phospholipids [19–21] and a reduction in the amount of SP-A [19, 21], have been observed during acute bacterial pneumonia. These changes have been associated with deleterious effects on lung function, e.g. significant decreases in the total lung capacity and arterial oxygen tension (P_{aO_2}) [22]. The inflammatory responses in the lungs together with endogenous

repair and defence mechanisms may have disturbed the synthesis, secretion or recycling of pulmonary surfactant and eventually resulted in an affected bronchoalveolar surfactant system [22].

Interestingly, total alveolar phospholipid content was not reduced in our patients. This is in contrast to the observations made in severe pneumonia, where a reduced phospholipid mass may be found [15, 21, 23]. Assuming that similar alveolar volumes have been assessed, these data indicate no substantial lack of total surfactant mass in the lung areas lavaged. The reduced ratio of the less surface active SA to the surface active LA indicates substantial amounts of LA, however the surface activity of this material was significantly reduced in CF. As no differences in the ability to convert large surfactant aggregates into small ones by *in vitro* surface area cycling were found, there is no indication of major changes in the activity of the intra-alveolar convertase [13, 14].

Besides biochemical abnormalities of surfactant, the inhibition of its function by proteins and other compounds gaining access to the alveolar space secondary to severe lung injury [6] is another well-established factor leading to disturbances in pulmonary surfactant. Although the inhibition of surfactant by transudated plasma is an attractive hypothesis to explain surfactant dysfunction, unequivocal evidence for this, and if so, its precise quantitative contribution, remains to be established for pneumonia and nosocomial infection [17, 21]. The data from this study do not support the view that surfactant inhibition in CF does play a major role, because when the water-soluble compounds were removed by lipid extraction, surface activity did not improve. In addition, the SA surfactant fraction from patients with CF was not more inhibitory at high protein concentration than the corresponding fraction from healthy controls. Lastly, the content of protein recovered was not different between CF and control patients. Additional mechanisms leading to impaired surfactant function, e.g. the activity of proteases or reactive oxygen species on the integrity and function of the surfactant associated proteins, may have been missed by the immunological assays used in the present study and need further investigation.

In this investigation, a higher number of neutrophils was associated with a worse surface activity ($p<0.05$, $r=0.678$, native surfactant at $3 \text{ mg}\cdot\text{mL}^{-1}$), a lower FEV₁ ($p<0.05$, $r=0.533$), but not with the biochemical parameters of the surfactant system. A higher surface activity of the surfactant isolated from CF patients was associated with an overall better clinical Berner score ($p<0.05$, $r=0.699$, native and lipid-extracted surfactant at $1 \text{ mg}\cdot\text{mL}^{-1}$), a higher FEV₁ ($p<0.003$, $r=0.862$, native surfactant at $3 \text{ mg}\cdot\text{mL}^{-1}$ and lipid-extracted surfactant at $1 \text{ mg}\cdot\text{mL}^{-1}$), and a higher FVC ($p<0.004$, $r=0.862$, native surfactant at $3 \text{ mg}\cdot\text{mL}^{-1}$ and lipid-extracted surfactant at $1 \text{ mg}\cdot\text{mL}^{-1}$). There was only a weak correlation to SP-A ($p=0.071$, $r=0.667$). Whether the severity of the inflammatory process in the CF lungs (represented here only by the percentage of neutrophils) or the overall clinical condition is primarily responsible for changes of surfactant has to be addressed in future studies that assess more variables of inflammation.

Our data and the small number of previous studies of surfactant in CF summarized in the introduction [7–10]

are in agreement with the view that surfactant dysfunction might contribute to lung disease in CF. A potential pathophysiological sequence may be suggested from comprehensive *in vitro* data and from animal experiments. In addition to alveolar stability during breathing, pulmonary surfactant will normally maintain the patency of the conducting airways [4, 5]. If not functioning properly, small amounts of liquid lining the inner wall of the airways will be attracted to the narrowest part of the airways, accumulate there and during expiration a liquid column will form and block the airway completely. The resulting obstructive air flow limitation may be partly reversible, *e.g.* with the application of positive expiratory pressure (PEP). Poorly ventilated lung tissue may become atelectatic and support the spread of the chronic inflammatory processes. However, it is clear that only well-designed clinical trials of exogenous surfactant application will definitely clarify the functional relevance of the observed impaired surfactant in CF.

The present investigation did not address other potential aspects of surfactant in CF. These include its role in improving the surface and transport properties of mucus [24], and an enhancement of bronchotracheal mucociliary clearance [25]. Finally, pulmonary surfactant modulates the function of immune cells in the lungs and participates in the nonspecific first-line host defence reactions [26].

In conclusion, these data demonstrate an impairment of bronchoalveolar surfactant in stable patients with cystic fibrosis. The reduced content of surface active components, such as phospholipids and surfactant protein A, were the most likely causes of reduced surface activity, rather than the inhibition by nonsurfactant components. However, additional data are necessary to clarify which factors are responsible for surfactant impairment in cystic fibrosis. This knowledge is highly desirable for a rational design of exogenous surfactant replacement trials in cystic fibrosis lung disease.

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