Eur Respir J
1991, 4, 1245–1250

In vivo and *in vitro* inactivation of bovine surfactant by an anti-surfactant monoclonal antibody

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In vivo *and* in vitro *inactivation of bovine surfactant by an anti-surfactant monoclonal antibody. E.P. Eijking, D.S. Strayer, G.J. van Daal, R. Tenbrinck, T.A. Merritt, E. Hannappel, B. Lachmann.*

ABSTRACT: In this study the importance of a low-weight surfactant protein (11 kDa) is demonstrated by selectively blocking this protein with a monoclonal antibody. In adult rats respiratory failure was induced by repeated bronchoalveolar lavage to remove all pulmonary surfactant. It was shown that surfactant mixed with the antibody was not capable of restoring lung function when compared with surfactant alone or surfactant mixed with control serum. Using the pulsating bubble surfactometer, it could be demonstrated that surfactant mixed with this antibody had a significant higher minimum surface tension when compared with surfactant alone, or surfactant mixed with an unrelated mouse immunoglobulin G (IgG). The inhibition of surfactant function by the monoclonal antibody suggests the importance of the 11 kDa protein for normal surfactant function.

Eur Respir J., 1991, 4, 1245–1250.

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Keywords: Animal model; antibody; blood gases; surfactant; surfactant associated proteins; surface tension.

Received: June 5, 1990; accepted after revision June 10, 1991.

This work was financially supported by the Dutch Foundation for Medical Research (SFMO) and, in part, by grant FDA-R-000112 from the US FDA.

Pulmonary surfactant is mainly composed of specific phospholipids and proteins. Surfactant proteins have been divided into three groups: SP-A, SP-B and SP-C (for review, see [1, 2]). Recently the presence of a collagenous protein, SP-D (MW 43 kDa, reduced), has been demonstrated in primary type II cell cultures. This protein has been shown to be immunologically and structurally different from SP-A and the low molecular weight surfactant proteins. The function of SP-D is still unknown [3].

SP-B and SP-C are low-weight hydrophobic proteins with overlapping molecular weights between 5 kDa and 18 kDa, depending on the animal species, the method of preparation and the state of reduction (for review, see [2]). Mixtures of phospholipids and low-weight hydrophobic proteins reduce surface tension and increase the effectiveness of exogenously administered phospholipids in foetal rabbits, as measured by an increased lung compliance [4–6]. On the other hand, it has been demonstrated that monoclonal and polyclonal antibodies directed against surfactant can inhibit the function of surfactant *in vitro*, *i.e.* lowering surface tension and facilitating adsorption [7–10].

The role of hydrophobic surfactant proteins in surfactant function in adult animals remains unclear. In the present study, a monoclonal anti-surfactant antibody was raised in rats. This monoclonal antibody recognizes an 11 kDa surfactant protein. The function of this 11 kDa surfactant protein was investigated *in vivo* and *in vitro*, by selectively blocking its function with the monoclonal antibody (C1).

Materials and methods

Surfactant

The surfactant used in these experiments is a natural surfactant isolated from bovine lungs in basically the same manner as described previously [11]. It consists of approximately 83% phospholipids, 1% hydrophobic proteins (SP-B and SP-C), the remainder being other lipids such as cholesterol, glyceride and free fatty acids. There is no SP-A in this surfactant preparation.

Monoclonal antibody

Monoclonal antibodies against bovine surfactant were raised in rats. Procedures used to raise, absorb and assay rat polyclonal antisera have been described in

detail elsewhere [9, 12–15]. In brief, F344 rats received bovine surfactant emulsified in Freund's complete adjuvant (Difco) subcutaneously, followed by intravenous boosts. Antibody reactivity to surfactant was measured by enzyme-linked immunosorbent assay (ELISA) [16] using antibody to mouse immunoglobulin (Ig), conjugated to alkaline phosphatase (Cappel), and an automated ELISA reader (Dynatech). The animals showing high serum titres of antibody to bovine surfactant were boosted and their spleen cells fused to the nonsecreting rat myeloma line YB2/0 (ATCC). After growth in HAT (hypoxanthine + aminopterin + thymidine) containing medium for two weeks, positive wells were selected, subcloned and expanded. Resulting hybridomas were grown in RPMI-1640 with 10% normal horse serum (Hyclone). These hybridomas have been stable for over 100 passages *in vitro*. One of these hybridomas, C1, secretes the monoclonal antibody used in the present study. Using isotype-specific antiserum, this antibody was identified as IgG.

Western blotting

Western blot analysis of the specific protein reactivity of C1 was performed under reducing conditions according to procedures described elsewhere [17, 18], with several modifications. Bovine surfactant proteins were electrophoresed in parallel lanes using sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE). All gels were 15% acrylamide, 0.1% SDS and 4 M urea. We transferred surfactant containing slab gel proteins to Immobilon® (Millipore Corp.), prepared according to package insert. All transfers were accomplished in standard transfer buffer [18], supplemented with 0.1% SDS. Filters were then cut into strips ("Spaghetti Westerns") and exposed to the antibody-containing culture supernatant (diluted 1:10), washed, and then exposed to rabbit anti-mouse IgG (1:20) (Miles Immunochemicals). Finally antibody-treated filter strips were incubated with 10^5 cpm of ^{125}I-Staphylococcus protein A per strip and visualized by autoradiography. Protein sizing was accomplished by interpolating between the positions of known molecular weight markers run in adjacent lanes. These procedures revealed that C1 recognizes an 11 kDa protein (fig. 1).

Measurement of surface activity

The effect of the antibody on surface activity was studied *in vitro* using the pulsating bubble surfactometer, as described by ENHORNING [19].

In brief, the surfactometer used in these *in vitro* experiments measures the pressure gradient across the air-liquid interphase of a bubble in a 37°C water-enclosed sample chamber. The bubble size is viewed through a microscopic optic. The bubble pulsates between a maximal (0.55 mm) and a minimal (0.45 mm) radius at a rate of 20 cycles·min^{-1}. The surface tension is calculated according to the law of Young and LaPlace and expressed as milli Newton·meter^{-1} (mN·m^{-1}). In this study a bubble was formed to a maximal radius and 15 s later pulsation was started. A continuous tracing of pressure differences was made 1 min after bubble formation.

Fig. 1. – Western blot analysis of antigenic specificity of the C1 monoclonal antibody generated in rats. The procedures used are described in the text. The C1 antibody is directed to an 11 kDa surfactant protein, indicated by the arrow on the left. The markers indicating the positions of proteins with known molecular weight are shown on the right; these are: albumin (68 kDa), ovalbumin (43 kDa), chymotrypsinogen (23 kDa) and ribonuclease (RNase) (18 kDa).

There were three groups of samples for surface activity measurements. In the first group (n=5) the sample chamber of the surfactometer contained surfactant only. In the second group (n=5) surfactant was mixed with an unrelated mouse IgG (vol:vol = 1:1). In the third group (n=5) surfactant was mixed with the undiluted culture supernatant of the C1 hybridoma, containing the monoclonal antibody. For the mixing procedure a Vortex mixer was used for 30 s, followed by incubation for 30 min at 37°C with shaking. The final surfactant concentration was 5 mg phospholipid·ml^{-1} in all groups. The corresponding antibody concentration was approximately 200 ng·ml^{-1} in both the second and the third groups. Of these groups surface tension was calculated at minimum radius after 5 min of pulsation.

Animal model

The studies were performed in 28 male adult Wistar rats (body weight: 240–260 g), divided into four groups. Respiratory failure was induced by lung lavage as described by LACHMANN *et al.* [20]. In brief, all rats were anaesthetized with pentobarbital sodium (60 mg·kg^{-1}, *i.p.*), tracheotomized and paralysed with pancuronium bromide (0.5 mg·kg^{-1}, *i.m.*). A catheter was inserted into the carotid artery. Lungs were lavaged six times with 8 ml warm saline (37°C) to produce an arterial oxygen tension (Pao_2) <80 mmHg at the following ventilator settings: pressure-controlled ventilation, fraction of inspired oxygen (Fio_2)=1.0, ventilation frequency=30·min^{-1}, peak airway pressure=26 cmH_2O, positive end-expiratory pressure (PEEP)=6 cmH_2O and inspiratory/expiratory ratio=1:2. These ventilator settings were maintained throughout the observation period; a Servo Ventilator 900 C (Siemens-Elema, Solna, Sweden) was used.

Treatment began approximately 5 min after the last lavage. Surfactant was intratracheally instilled in three of four groups at a concentration of approximately 150 mg phospholipids·kg^{-1}. Group 1 (n=6) received 2 ml surfactant only; group 2 (n=7) received 2 ml surfactant mixed with the monoclonal antibody (C1) (vol:vol = 1:1); group 3 (n=6) received 2 ml surfactant mixed with the control preparation (RPMI-1640 + 10% horse serum in which YB2/0 cells alone had been cultured); Group 4 (n=9) received 2 ml saline only, intratracheally. The antibody in Group 2 was used as a culture supernatant at concentrations of 400–500 ng·ml^{-1} in RPMI-1640 + 10% normal horse serum. For Groups 2 and 3, surfactant plus culture supernatant or surfactant plus control preparation, were incubated together for 30 min at 37°C with shaking, as for the measurement of surfactant tension described above.

Blood samples were taken from the carotid artery of each rat just before and 5 min after bronchoalveolar lavage (directly followed by treatment) and at 5, 30, 60, 120 and 180 min post-treatment. Pao_2 and arterial carbon dioxide tension ($Paco_2$) were measured with the ABL 330 Acid-Base Laboratory (Radiometer, Copenhagen, Denmark).

Histological examination

At the end of the animal study the rats were sacrificed with an overdose of intra-arterial administered pentobarbital. The lungs were inflated to 20 cmH_2O with air, the trachea was clamped and the lungs removed and fixed in 10% formalin for light microscopic examination. The tissues were dehydrated and embedded in paraffin, and 6 μm sections of the base of the right lung were stained with haematoxylin and eosin.

Statistical analysis

Statistical evaluation of collected data was performed using the Mann-Whitney-Wilcoxon test. Statistical significance was accepted at $p<0.05$ (two-tailed).

Results

Measurement of surface activity

After 5 min of pulsation, minimum surface tension was high in the surfactant plus C1 sample (17.7±0.63 mN·m^{-1}), whereas the surfactant alone (4.3±0.76 mN·m^{-1}) and surfactant plus mouse IgG (3.1±0.67 mN·m^{-1}) samples had significantly lower minimum surface tensions; the difference between these latter sample groups was not significant.

Animal studies

Figure 2 shows Pao_2 values for each group. Before lavage Pao_2 values are high in all groups, whereas after lavage all values are low; the intergroup differences both before and after lavage are not statistically significant. After treatment, the difference in Pao_2 values between the surfactant plus C1 group and surfactant alone group remains highly significant throughout the observation period, as does the difference between the surfactant plus C1 group and surfactant plus RPMI-1640 group. The Pao_2 values of the surfactant only group are higher than those of the surfactant plus RPMI-1640 group, but the difference is not significant. Comparing Pao_2 values of the surfactant plus C1 group with the saline group revealed almost identical values after 60 min post-treatment.

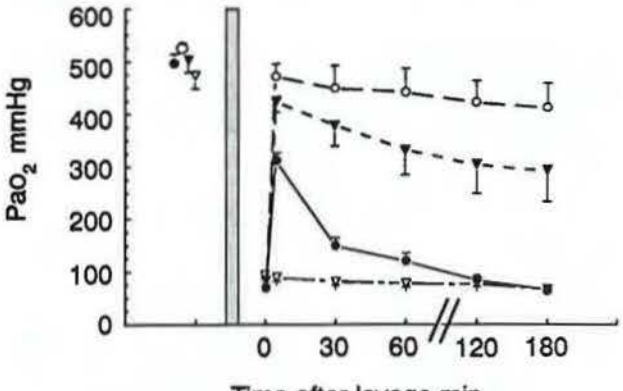

Fig. 2. – Pao_2 values in mmHg (mean±SEM) of the four treatment groups: surfactant plus C1 (●); surfactant only (○); surfactant plus RPMI-1640 (▼); saline only (▽). The vertical grey bar indicates the moment of broncholavage; t=0 indicates Pao_2 values 5 min after lavage, immediately followed by treatment. Pao_2: arterial oxygen tension.

Table 1 shows $Paco_2$ values for all groups; the intergroup differences before and 5 min after lavage are not significant. The $Paco_2$ values of the group receiving surfactant plus C1 are significantly higher at 60–180 min compared to the group receiving surfactant alone. The difference in values between the groups receiving surfactant plus C1 or surfactant plus RPMI-1640 is significant at 60 and 120 min post-treatment. As with Pao_2, the difference in $Paco_2$ values between the surfactant plus RPMI-1640 and surfactant alone groups is not significant at any time. Similarly, there is no significant difference between post-lavage $Paco_2$ values in the surfactant plus C1 group, compared to the saline alone group.

Table 1. – $Paco_2$ values in mmHg (mean±SEM) before and after lavage and after different therapeutic regimes

	Surfactant n=6	Surf. + C1 n=7	Surf. + RPMI-1640 n=6	Saline n=9
Before lavage	37.6±1.84	34.7±1.60	39.0±2.10	39.4±3.30
5 min after lavage	57.1±5.76	59.7±2.85	56.2±4.81	51.3±3.77
5 min after treatment	44.3±3.08	50.1±2.30	46.1±1.79	51.5±3.20
30 min	42.7±3.41	46.6±2.41	40.5±1.69	51.8±3.62*
60 min	37.5±2.82	45.6±1.93*	39.2±1.55	52.0±3.79*
120 min	37.7±1.97	47.8±1.98*	39.3±2.48	51.8±3.98*
180 min	39.9±2.99	51.4±3.35*	45.0±3.71	56.6±4.19*

*: statistically significant difference between surfactant and the other groups; for further significant differences see text. $Paco_2$: arterial carbon tension.

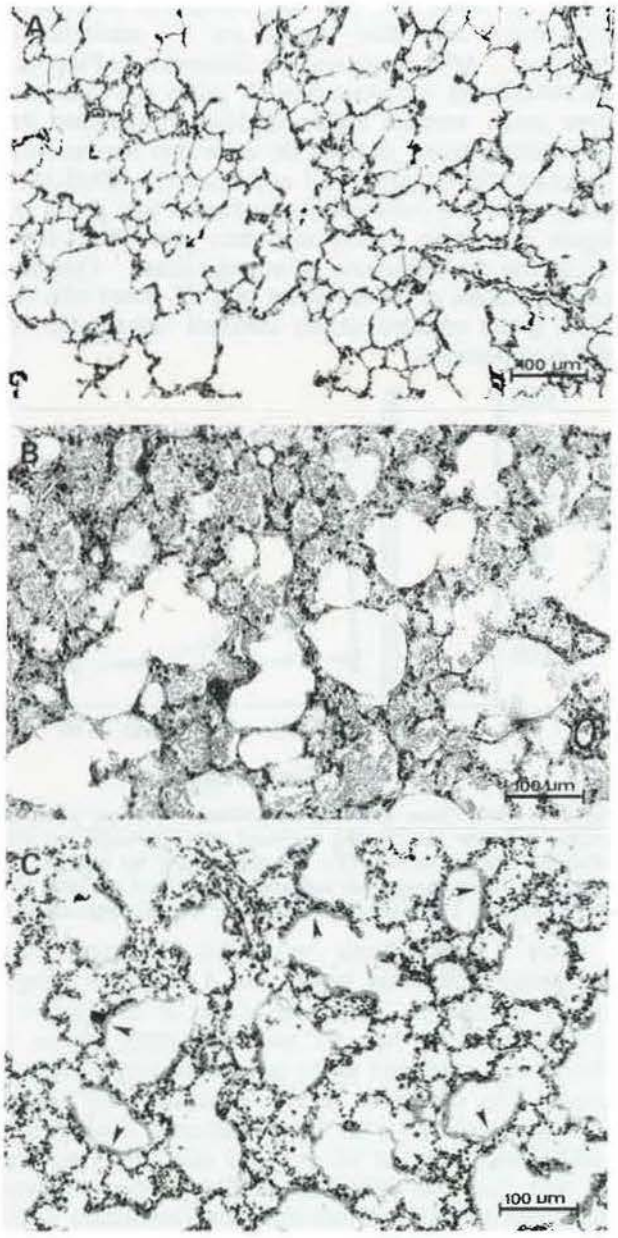

Fig. 3. – Typical histological findings in rats: A) after treatment with surfactant plus RPMI-1640; B) saline; C) after treatment with surfactant plus C1 (arrows indicate the eosinophilic substance covering the alveolar walls). Haematoxylin and eosin stain.

Light-microscopic findings

The lungs of the animals receiving surfactant only or surfactant plus RPMI-1640 were well-aerated (fig. 3A). The lungs from animals receiving saline showed widespread atelectasis, alternating with areas of hyperexpansion; the alveoli were filled with amorphous exudate and erythrocytes, indicating damage to the alveolar-capillary memrane (fig. 3B). Lungs of rats which received surfactant plus C1 showed regions with atelectasis, alternating with aerated areas, which were partly overdistended. These overdistended alveoli were covered with an eosinophilic substance. A typical light-microscopic picture is shown in figure 3C.

Discussion

It has been reported that in adult animal models (guinea-pigs, rabbits, pigs and dogs) repeated bilateral whole-lung lavage with saline severely impairs lung function through loss of lung surfactant [20]. It is established that this lung lavage model is useful for a variety of experimental purposes, including, *e.g.* testing of different surfactant preparations, and to demonstrate that exogenous surfactant restores blood gases to normal [21–24]. Since the purpose of this study was to investigate the influence of a monoclonal antibody on the function of exogenous surfactant containing the hydrophobic surfactant proteins (SP-B and SP-C), the lung lavage model was used to detect any inhibiting effect of this monoclonal antibody on the effectiveness of the exogenous surfactant preparation to restore blood gases.

The monoclonal antibody used in this study, C1, recognizes an 11 kDa surfactant associated protein. TANAKA *et al.* [25] have demonstrated that bovine surfactant contains SP-B with a molecular weight of 10 kDa in the reduced state. SP-C has a much lower molecular weight after reduction (for review, see [2]). These results and the findings reported by others suggest that the 11 kDa protein is most probably a bovine SP-B monomer.

This C1 monoclonal antibody inhibits pulmonary surfactant function, as demonstrated by low Pao_2 and high surface tension in this study. A similar elevation of the minimum surface tension of porcine surfactant

after mixing with a monoclonal antibody against porcine SP-B has been reported by KOBAYASHI *et al.* [26]. They also demonstrated that this mixture of porcine surfactant and monoclonal antibody, unlike porcine surfactant alone, is not able to improve lung compliance in immature rabbit foetuses. The reason for this inhibition of surfactant function could be that the monoclonal antibody simply inhibits the function of SP-B. As already mentioned in the introduction, the hydrophobic proteins SP-B and SP-C play an essential role in the function of surfactant [4–6].

Another mechanism by which the antibody perhaps inhibits surfactant may lie in the formation of surfactant-antibody aggregates, with subsequent loss of surfactant function. Comparable results have been reported in an *in vitro* study using IgG subfragments where it was found that surfactant mixed with polyclonal rabbit anti-surfactant antiserum inhibited the ability of surfactant to lower surface tension, as measured by the pulsating bubble surfactometer. It was demonstrated that bivalent antibody ($F[ab']_2$) preparations inhibited surfactant activity, whereas monovalent antibody (F[ab']) fragments did not [9]. Thus, it was concluded that these polyclonal antibodies agglutinate surfactant. It is possible that this aggregation of surfactant with the C1 monoclonal antibody alters the surface activity of surfactant and/or that the aggregates themselves are responsible for further deterioration of pulmonary function.

Another explanation for the failure of surfactant mixed with the monoclonal antibody to restore lung function could be that the antibody causes a configuration change of the 11 kDa protein, which affects the protein-phospholipid interaction. Also, it is possible that other physiochemical characteristics of surfactant, such as charge, are altered. These changes in characteristics of surfactant could affect the function of surfactant, *i.e.* surface tension lowering capacity.

The reason for the initial increase of Pao_2 after administration of surfactant plus the monoclonal antibody followed by a decrease of Pao_2 (fig. 2), could be explained as a functional surfactant deficit. In a recent study (unpublished observations), using the same lung lavage model, the effect of different doses of surfactant, ranging from 10–300 $mg \cdot kg^{-1}$, to improve arterial oxygenation was investigated. In adult rats receiving only 10–15 mg surfactant-phospholipids$\cdot kg^{-1}$ surfactant intratracheally, a similar behaviour to that observed in the present study could be demonstrated, namely, an initial rise of Pao_2, rapidly followed by deterioration of lung function. Rats receiving the same dose of surfactant used in the present study (150 mg surfactant-phospholipids$\cdot kg^{-1}$) showed the same results as the rats receiving surfactant alone in this study, *i.e.* restoration of blood gases to normal values and remaining constantly high for the observed period. From these findings one could conclude that about 90% of the exogenous surfactant was inhibited by the monoclonal antibody and perhaps, in part, by the horse serum. The amount of surfactant which was not inhibited by the monoclonal antibody was too low to stabilize the lungs at the applied ventilator settings for the observed period.

As can be seen figure 2, the Pao_2 values of rats receiving surfactant plus control serum (RPMI-1640 + 10% horse serum) are lower than Pao_2 values of rats receiving surfactant alone, although this difference is not significant. An explanation for the lower Pao_2 values in the group receiving surfactant plus control serum could lie in the fact that proteins in the control serum inhibited a part of the exogenous surfactant. This inhibitory capacity of different serum proteins has already been demonstrated in *in vitro* studies [27, 28].

Histologically, lungs of rats receiving surfactant plus the monoclonal antibody showed an eosinophilic substance covering the alveolar walls. This finding could suggest that the C1 antibody aggregates surfactant by crosslinking 11 kDa surfactant proteins, supporting the suggestion that antibody inactivates surfactant by causing aggregation of surfactant-antibody complexes. On the other hand, it is possible that the histological findings reflect the presence of hyaline membranes due to fibrin deposition, caused by leakage of the alveolar-capillary membrane as a result of a functional surfactant deficit. No proofs were performed in this study to demonstrate the presence of fibrin in the material covering the alveolar walls; but in earlier histological studies hyaline membranes were observed in animal lungs following lung lavage and ventilation for a period of 2–6 h [20, 23]. The lungs of rats receiving saline showed widespread atelectasis and oedema-filled alveoli. The reason for the difference between the lungs of rats receiving surfactant plus C1 and lungs of rats receiving saline alone could perhaps be explained by the initial protective effect of surfactant in the first group. Perhaps there was still some partial protective effect from surfactant remaining, preventing excessive intra-alveolar oedema accumulation, although this effect was not enough to permanently increase Pao_2. When compared to lungs of rats receiving surfactant plus RPMI-1640, lungs of rats receiving surfactant plus C1 showed regions with atelectasis and an eosinophilic substance covering the aerated alveoli.

In conclusion, we have demonstrated that a monoclonal anti-surfactant antibody (C1) to an 11 kDa apoprotein of bovine surfactant (SP-B) inhibits the function of exogenous bovine surfactant, both *in vivo* and *in vitro*. As already suggested in other studies [4–6], these results confirm the importance of the hydrophobic surfactant proteins for optimal surfactant function in the adult lung.

Acknowledgement: The authors thank L. Visser-Isles for English language editing.

References

1. Hawgood S. – Pulmonary surfactant apoproteins: a review of protein and genomic structure. *Am J Physiol*, 1989, 257, L13–L22.
2. Possmayer F. – A proposed nomenclature for pulmonary surfactant-associated proteins. *Am Rev Respir Dis*, 1988, 138, 990–998.

3. Persson A, Chang D, Rust K, Moxley M, Longmore W, Crouch E. – Purification and biochemical characterization of CP4 (SP-D), a collagenous surfactant-associated protein. *Biochemistry*, 1989, 28, 6361–6367.
4. Revak SD, Merritt TA, Degryse E, Stefani L, Courtney M, Hallman M, Cochrane CG. – Use of human surfactant low molecular weight apoproteins in the reconstitution of surfactant biologic activity. *J Clin Invest*, 1988, 81, 826–833.
5. Yu S-H, Possmayer F. – Reconstitution of surfactant activity using the 6-K apoprotein associated with pulmonary surfactant. *Biochem J*, 1986, 236, 85–89.
6. Whitsett JA, Ohning BL, Ross G, Meuth J, Weaver T, Holm BA, Shapiro DL, Notter RH. – Hydrophobic surfactant-associated protein in whole lung surfactant and its importance for biophysical activity in lung surfactant extracts used for replacement therapy. *Pediatr Res*, 1986, 20, 460–467.
7. Merritt TA, Stayer DS, Hallman M, Spragg RD, Wozniak P. – Immunologic consequences of exogenous surfactant administration. *Semin Perinatol*, 1988, 12, 221–230.
8. Strayer DS, Merritt TA, Spragg R, Hallman M. – Immunogenicity of surfactant and its implications for replacement therapy. *In:* Surfactant replacement therapy in neonatal and adult respiratory distress syndrome. B. Lachmann ed., Springer-Verlag, Berlin, Heidelberg, 1988, pp. 301–313.
9. Strayer DS, Merritt TA, Makunike C, Hallman M. – Antigenicity of low molecular weight surfactant species. *Am J Pathol*, 1989, 134, 723–732.
10. Strayer DS, Hallman M, Merritt TA. – Immunogenicity of surfactant: I. Human alveolar surfactant. *Clin Exp Immunol*, 1991, 83, 35–40.
11. Metcalfe IL, Enhorning G, Possmayer F. – Pulmonary surfactant-associated proteins: their role in the expression of surface activity. *J Appl Physiol: Respirat Environ Exercise Physiol*, 1980, 49, 34–41.
12. Strayer DS, Merritt TA, Lwebuga-Musaka J, Hallman M. – Surfactant-anti-surfactant immune complexes in infants with respiratory distress syndrome. *Am J Pathol*, 1986, 122, 353–362.
13. Kennet RH. – Fusion protocols. *In*: Monoclonal antibodies. R.H. Kennet, T.J. McKearn, K.B. Bechtol eds, Plenum Press, New York, 1980, pp. 365–367.
14. McKearn TJ. – Cloning of hybridoma cells by limiting dilution in fluid phase. *In:* Monoclonal antibodies. R.H. Kennet, T.J. McKearn, K.B. Bechtol eds, Plenum Press, New York, 1980, p. 374.
15. Jonak ZL. – Isolation of monoclonal antibodies from supernatant by $(NH_4)_2SO_4$ precipitation. *In*: Monoclonal antibodies. R.H. Kennet, T.J. McKearn, K.B. Bechtol eds, Plenum Press, New York, 1980, pp. 405–406.
16. Engvall E, Pesce A (eds). – Quantitative enzyme immunoassay. *Scand J Immunol*, 1978, 8 (Suppl. 7).
17. Maizel JV Jr. – Gel electrophoresis of proteins. *In*: Methods in virology, vol. 5. K. Maramorosch, H. Koprowski eds, Academic Press, New York, 1971, pp. 179–246.
18. Towbin H, Staheiln T, Gordon J. – Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some applications. *Proc Natl Acad Sci USA*, 1979, 76, 4350–4354.
19. Enhorning G. – Pulsating bubble technique for evaluating pulmonary surfactant. *J Appl Physiol: Respirat Environ Exercise Physiol*, 1977, 43, 198–203.
20. Lachmann B, Robertson B, Vogel J. – *In vivo* lung lavage as an experimental model of the respiratory distress syndrome. *Acta Anaesthesiol Scand*, 1980, 24, 231–236.
21. Lachmann B, Fujiwara T, Chida S, Morita T, Konishi M, Nakamura K, Maeta H. – Improved gas exchange after tracheal instillation of surfactant in the experimental adult respiratory distress syndrome. *Crit Care Med*, 1981, 9, 158.
22. Lachmann B, Fujiwara T, Chida S, Morita T, Konishi M, Nakamura K, Maeta H. – Surfactant replacement therapy in the experimental adult respiratory distress syndrome (ARDS). *In*: Pulmonary surfactant system. E.V. Cosmi, E.M. Scarpelli eds, Elsevier Science Publishers, Amsterdam, 1983, pp. 231–235.
23. Berggren P, Lachmann B, Curstedt T, Grossmann G, Robertson B. – Gas exchange and lung morphology after surfactant replacement in experimental adult respiratory distress syndrome induced by repeated lung lavage. *Acta Anaesthesiol Scand*, 1986, 30, 321–328.
24. Kobayashi T, Kataoka H, Ueda T, Murakami S, Takada Y, Kokubo M. – Effects of surfactant supplement and end-expiratory pressure in lung lavaged rabbits. *J Appl Physiol: Respirat Environ Exercise Physiol*, 1984, 57, 995–1001.
25. Tanaka Y, Takei Kanazawa Y. – Lung surfactants. II. Effects of fatty acids, triacylglycerols and protein on the activity of lung surfactant. *Chem Pharm Bull (Tokyo)*, 1983, 31, 4100–4109.
26. Kobayashi T, Nitta K, Takahashi R, Kurashima K, Robertson B, Suzuki Y. – Activity of pulmonary surfactant after blocking SP-A and SP-B. *J Appl Physiol*, 1991, (in press).
27. Fuchimukai T, Fujiwara T, Takahashi A, Enhorning G. – Artificial pulmonary surfactant inhibited by proteins. *J Appl Physiol*, 1987, 62, 429–437.
28. Holm BA, Enhorning G, Notter RH. – A biophysical mechanism by which plasma proteins inhibit lung surfactant activity. *Chem Phys Lipids*, 1988, 49, 49–55.

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RÉSUMÉ: Cette étude démontre l'importance de la protéine du surfactant de bas poids moléculaire (11 kDa) au moyen d'un blocage sélectif de cette protéine par un anticorps monoclonal. Chez les rats adultes, une insuffisance respiratoire a été induite par lavages broncho-alvéolaires répétitifs, de façon à prélever tout le surfactant pulmonaire. Il a été démontré que le surfactant mêlé à l'anticorps n'était pas capable de restaurer la fonction pulmonaire, par comparison avec le surfactant seul ou avec le surfactant mêlé à un sérum de contrôle. En utilisant le surfactomètre à bulbe pulsatile, il a pu être démontré que le surfactant mêlé à l'anticorps avait une tension de surface minimale significativement plus élevée par comparison avec le surfactant seul ou avec le surfactant mêlé à une IgG de souris sans relation. L'inhibition de la fonction du surfactant par les anticorps monoclonaux suggère l'importance de la protéine 11 kDa du surfactant pour une fonction normale de ce dernier.
Eur Respir J., 1991, 4, 1245–1250.