Übersichten

Bronchoalveolar Lavage Proteins*

B. Müller and P. von Wichert
Medizinische Poliklinik, Philipps Universität Marburg

Summary. Since the discovery of the extra-cellular lining material of the lung and the possibility harvesting this source by endobronchial lavage this material has been the object of many studies directed to analyze its components, function and possible change in the diseased lung.

The best known component of the extra-cellular lining material is the phospholipid and its fatty acid composition. But also on the cellular material much emphasis has been taken with the aim using its cytology as diagnostic parameter. However, very few informations were obtained about the protein material also washed out during the endobronchial lavage.

As it was demonstrated by immunological methods the proteins of the extra-cellular lining material consist of serum identical proteins and those being obviously specific for the lung tissue. As found, most serum identical proteins occure in the same amounts as found in the blood serum, and the molecular weight in general range up to 160,000 daltons indicating that there must be a restriction in passage of high molecular weight proteins through the lumen walls of the endothelium. Some proteins, IgG, IgA, do occur in a higher level in the extra-cellular lining material leading to the suggestion that these proteins were synthesized and secreted by the lung tissue itself. – The molecular weight of the lung specific proteins range from 16,000-340,000 daltons. Under reducing conditions however, for all species listed, two classes of subunits ~36,000 and 12,000 daltons – result, indicating that these proteins might have comparable functions in the different species. The exact function of these specific proteins as well as the serum identical proteins till now is not known. One can only speculate that the serum identical proteins will have the same function in plasma and the lung, and that the specific proteins are involved in the formation in the surfactant system. Different amounts of lung specific proteins in lavage of disease lungs suggest that their estimation might be an additional useful parameter in diagnosis of lung diseases.

Key words: Bronchoalveolar lavage – Extra-cellular lining material – Serum identical proteins – Lung specific proteins

The cellular lining of the lung alveoli covering the epithelium of the alveoli and the terminal bronchi is lined with a highly surface-active material, the "pulmonary surfactant" which was first discovered by von Neergaard [65]. This material, known to prevent lung collaps during expiration [58] could be washed out from the lungs by endobronchial lavage, and has been the object of many studies directed to analyze the components, their function and their possible change in the diseased lung [16, 19, 20, 23, 30, 31, 42, 43, 60, 61, 68].

The clinical importance of surfactant was demonstrated in the end fifties [1, 2, 29] by observations that infants, died from the respiratory distress syndrome, were deficient in surfactant. – Also in animal experiments an altered function of surfactant was reported [33, 35, 36, 55, 79]. On the other hand, in animal and human experiments after synthetic surfactant replacement therapy the surfactant function had been restored [57, 63, 74].

The surfactant is composed of phospholipid and protein components, perhaps acting in molecular interactions. This interaction, however, is not

* Supported by the Deutsche Forschungsgemeinschaft Wi 359/7+8

Abbreviation: ARDS = adult respiratory distress syndrome
well understood, although it is known that at least some of the proteins have the quality of apoproteins perhaps similar to those of the serum.

The best known component of the surfactant system is the phospholipid, the individual phospholipid composition as well as the fatty acid composition [4, 17, 22, 26, 27, 45, 75].—Also on the cellular material much emphasis has been taken with the aim using its cytology as diagnostic parameter [34, 54].

However, very little informations were obtained about proteins, also washed out by endobronchial lavage. This is astonishing the more since 20–30% by weight, varying from species to species, of the surfactant is protein material. Since in the last decade several studies were done on proteins, this brief paper was attempted to summarize the current state of investigation in the field of bronchoalveolar lavage proteins.

**Serum Identical Proteins**

As reported by several investigators serum identical proteins are found in the bronchoalveolar lavage of the human [13, 14, 72, 73] and laboratory animals [56, 71]. In an extensive study Bell and co-workers [6] showed for the human 23 serum identical antigens (Table I). As shown in own experiments [64] and also by others most serum proteins occure in the same distribution as measured in the blood serum [6, 28, 70, 83, 85]. The molecular weight of the serum identical proteins found in the bronchoalveolar lavage range up to 160,000 daltons indicating that there is a restriction in passage of high molecular weight proteins [5, 6]. The striking observation that IgG and IgA do occure in a higher level in bronchoalveolar lavage than in the serum is a result of the secretion of IgA, and perhaps also IgG, by lung tissue as components of the secretory immunological system of the mucosa [84]. These immunoglobulins were synthesized by lung tissue [32] and could be identified histologically in the mucosa and the extra-cellular lining of the lung [14, 15]. If there is additionally a selective transport of proteins across the mucosa-membrane similar to that across the placenta [84, 86] is not known.

In clinical trials it was attempted to determine the amount of particular proteins in correlation to total protein amount with the aim to use that correlation as diagnostic parameters. However, the concentration of serum-identical proteins in bronchoalveolar lavage could be influenced by artefacts. The occasional detection of alpha-2-macroglobulin in the bronchoalveolar lavage might be the result of macrophage-activity, because these cells synthesize and secrete this protein [87]. Moreover, it could not be excluded that also other cells originally not derived from the lung, could secrete serum identical proteins.

An other striking report was given by Reifenrath and Zimmermann [71], who compared the albumin content of normal proceeded bronchoalveolar lavage fluid with such, obtained by micropuncture of the alveoli; because only small amounts of the serum albumin were found in the micropunctate they concluded that the content of serum proteins will be the result of blood contamination during the lavage procedure. Therefore until now the estimation of the concentration of serum-identical proteins in lavage has to be questioned and further studies must show how to quantify the proteins in lavage material before using them as a mirror of alveolar alterations. Methods with dilution techniques are not yet satisfactory [3].

**Table 1. Serum identical proteins in lavage effluents from the lungs of healthy humans**

| Protein                  | % of Total (mean ± SEM) |
|--------------------------|-------------------------|
| IgG                      | 19.0 ± 1.7              |
| IgA                      | 10.2 ± 1.0              |
| IgM                      | 0.08 ± 0.04             |
| IgD                      | 0 ± 0                   |
| IgE                      | 0 ± 0                   |
| 2-1-Lipoprotein          | 0.34 ± 0.18             |
| a2-Macroglobulin         | 0.18 ± 0.03             |
| Fibrinogen               | 0.38 ± 0.03             |
| C3                       | 0.98 ± 0.09             |
| Ceruloplasmin            | 0.38 ± 0.03             |
| Plasminogen              | 0 ± 0                   |
| C-reactive protein       | 0 ± 0                   |
| Haptoglobin              | 0.96 ± 0.20             |
| Transferrin              | 5.6 ± 0.3               |
| Hemopexin                | 0.85 ± 0.09             |
| Albumin                  | 52.5 ± 2.5              |
| Prealbumin               | 0.09 ± 0.01             |
| a1-Antitrypsin           | 3.5 ± 0.3               |
| Gc-globulin              | 0.24 ± 0.02             |
| a2-HS-glycoprotein       | 0.65 ± 0.06             |
| a2-Acid glycoprotein     | 0.74 ± 0.12             |
| β2-Glycoprotein 1        | 0.16 ± 0.02             |
| Total                    | 95.6                    |

* according to Bell et al. [6] from George and Hook [25]  
* n=23
experimental data until now. IgG and IgA probably protect the lung from virus and bacterial infection and also for transferrin an anti-bacterial effect was claimed [37, 47, 66, 69]. The alpha-1-antiproteinase is thought to prevent lung damage due to protease release from inflammatory cells [24]. This function is very important in lung diseases like emphysema or ARDS [23]. However, the relationship of these serum-identical proteins to the extra-cellular lining material concerning the surface tension lowering function till the current state of investigation is unknown.

Lung Specific Lavage Proteins

The question of whether specific extra-cellular lining proteins exist has induced a lot of work and in the meantime several reports were given, describing specific lavage proteins [8, 18, 21, 38-40, 44, 46, 48-52, 59, 62, 80, 81, 88]. The studies were mostly done on a variety of laboratory animals and only few reports were given for the human specific lavage proteins in the epithelial lining material [9, 10, 12, 77, 78]. The same was true for the human amniotic fluid representing a permanent bronchoalveolar lavage in the embryo [11, 41, 48].

One problem in the field of specific proteins of the extracellular lining material is however, that there are several different methods for the preparation of these proteins. It is obvious that different preparations lead to different results [64], and therefore it is difficult to describe all specific proteins for every laboratory animal.

Generally in the literature the specific lavage proteins were claimed as "apoproteins" because they do occur in preparations that have a surface lowering activity on a surface balance in-vitro [44]. In only few cases it was demonstrated, that the proteins possess a phospholipid-binding ability [53, 89]. Particularly for the human such an ability was never clearly demonstrated.

Despite the various different methods for the preparation some comparable lung specific lavage antigens were demonstrated for the human and laboratory animals. Out of them two proteins with molecular-weights, under reducing conditions, of about 36,000 daltons and 12,000 daltons have reached a general interest. If lung specific proteins with a higher molecular weight were subjected to reducing conditions in nearly all experiments the 36,000 and 12,000 daltons subunits were found (Table 2). These observations perhaps indicate a similar function of these two proteins in the different species. There is no immunological cross-reaction between the anti-sera and the 36,000 and 12,000 daltons proteins in different laboratory animals, except between human and monkey [78]. As Table 2 shows most of the studies were performed on these proteins while for proteins with a higher molecular-weight only a few reports exist.

Functional and Clinical Aspects of the Specific Lavage Proteins

Studies on bronchoalveolar lavage from patients with alveolar proteinosis indicated that the 36,000 daltons protein is present in a higher level in these patients than in the bronchoalveolar lavage from healthy non-smoking volunteers. This protein has been also detected in the amniotic fluid and increases in concentration in the amniotic fluid with advancing gestational age [77]. Establishing an enzyme-immuno-assay the 36,000 daltons protein in amniotic fluid has been used to determine the maturity of the fetal lung. The results are well correlated to the common lecithin-sphingomyeline ratio and the appearance of phosphatidylglycerol. Particularly in diabetic pregnancy the estimation of this specific protein is a better predictor for fetal lung maturity than the common used parameters [41]. In experiments in rats with alloxan diabetes mellitus the 12,000 daltons protein was shown to decrease in comparison with the normal control [81]. It was speculated that this protein is a glycoprotein which is insufficiently synthesized in diabetes mellitus lung as it is known for carbohydrate and the phospholipids. One may speculate that also in other lung diseases the estimation of specific proteins of the extra-cellular lining material may be helpful as additional diagnostic parameter.

Our knowledge about the precise function of these proteins unfortunately is rather incomplete. Most of the proteins were identified as being associated with phospholipids [53, 89], and considered therefore to be important for a rapid film generation at the air-liquid-interface in the alveoli by providing a lipid protein arrangement together with calcium ions and including phosphatidylcholine and phosphatidylglycerol [82]. However, the nature of these protein-ion-lipid interaction is unknown but it is suggested that these aggregations may reduce the activation-energy for absorption and/or spreading of the phospholipids at the air-liquid-interface [7].

Doubtless our understanding of the function of the specific proteins of the extra-cellular lining in the alveoli and the terminal bronchi is limited. Concerning the important role of lowering the surface tension in the alveoli for preventing alveolar
Table 2. Lung specific proteins from different sources of the human and laboratory animals

| Species | Origin          | Molec. weight native | Molec. weight reduced | Additional characteristics | Involvement/Function                  | Reference No. |
|---------|-----------------|----------------------|-----------------------|---------------------------|--------------------------------------|---------------|
| Human   | Lavage 80,000   | 62,000               | 36,000 26,000 16,000 | tryptic degradation       | in alveolar proteinosis              | [12]          |
|         | 250,000         |                      |                       | PAS pos.                  | enhanced in alveolar proteinosis     | [76]          |
|         | 62,000 36,000   |                      |                       | PAS pos. PAS pos.         | in alveolar proteinosis              | [67]          |
|         | 62,000 36,000   |                      |                       | PAS pos.                  |                                     | [77]          |
|         | lung tissue     | 400,000              | 34,000                | PAS pos.                  |                                     |               |
|         | homogenate 20,000 |                    |                       | PAS pos.                  |                                     |               |
|         | amniotic fluid  | 62,000               | 36,000                | PAS pos.                  |                                     | [11]          |
| Rat     | Lavage 160,000  | 38,000               | 32,000                | in Typ II cells           |                                     | [39, 40]      |
|         | 140,000 78,000  |                      |                       | in diabetes mellitus      |                                     |               |
|         | 110,000         |                      |                       | content decreased         |                                     |               |
|         | Typ II cells    | 35,000               | 10,000                | PAS pos.                  | DPPC-binding                         | [52, 80]      |
|         | 35,000          |                      |                       | PAS pos.                  |                                     |               |
|         | 72,000 34,000   |                      |                       | PAS pos.                  | DPPC-binding                         | [21, 51]      |
|         | 70–80,000       | 35–40,000            | 10–12,000             | PAS pos.                  |                                     |               |
|         | 72–73,000       | 35–45,000            | 10–12,000             | PAS pos.                  |                                     |               |
| Dog     | Lavage 36,000   |                      | 10,000                | Lipid binding             |                                     | [49]          |
|         | 70–80,000       | 35–45,000            | 10–12,000             | DPPC-binding              |                                     | [51, 52]      |
| Rabbit  | Lavage 340,000  |                      |                       | PAS pos.                  |                                     | [59]          |
|         | Lavage          | 62,000               |                       | PAS pos.                  |                                     | [8]           |
|         | Lavage bodies   | 36,000               |                       | PAS pos./IEP 7.4          |                                     |               |
| Sheep   | Lavage 34,000   |                      |                       | PAS pos.                  |                                     | [46]          |
|         | Lavage 120,000  | 35,000               | 30,000 10,000         | Lipid binding             |                                     | [21]          |
| Chicken | 62,000 35,000   |                      |                       | PAS pos.                  |                                     | [8]           |

Collaps and fluid overload research in this particular field would be important for a better understanding of lung structure and function in health and disease. More work on biochemical background and physiological function is needed and we like to emphasize too, the need of clinical trials to establish the diagnostic value of the lung specific proteins in lung diseases. One may speculate that this may lead to diagnostic tools like those used to determine lung maturity in amniotic fluid.

Acknowledgement: The excellent technical assistance of Mrs. Ch. Skurk is gratefully acknowledged.

References

1. Adams FJ, Fujiwara T, Emmanouilides G, Scudder A (1965) Surface properties and lipids from lungs of infants with hyaline membrane disease. J Pediatr 66:357–364
2. Avery ME, Mead J (1959) Surface properties in relation to atelectasis and hyaline membrane disease. Am J Dis Child 97:517–523
3. Baughman RP, Bosken CH, Loudon RG, Hurtubise P, Wessler T (1983) Quantitation of bronchoalveolar lavage with methylene blue. Am Rev Respir Dis 128:266-270
4. Baxter CF, Rouss R, Simon G (1972) Variations among vertebrates of lung phospholipid class composition. Lipids 4:243-244
5. Bell DY, Hook GER (1979) Pulmonary alveolar proteinosis: analyses of airway and alveolar proteins. Am Rev Respir Dis 119:979-900
6. Bell DY, Haseman JA, Spock A, McLennan G, Hook GER (1981) Plasma proteins of the bronchoalveolar surface of the lungs of smokers and nonsmokers. Am Rev Respir Dis 124:72-79
7. Benson BJ, Williams MC, Hawgood S, Sargeant T (1984) Role of lung surfactant-specific proteins in surfactant structure and function. In: von Wichert P (ed) Current Concepts in Surfactant Research. Karger, Basel München Paris pp 93-92
8. Bhattacharyya SN, Passero MA, DiAugustine RP, Lyn
9. Bell DY, Hook GER (1979) Pulmonary alveolar proteinosis: analyses of airway and alveolar proteins. Am Rev Respir Dis 119:979-900
10. Bhattacharyya SN, Lynn WS (1977) Studies on structural and function. In: von Wichert P (ed) Current Concepts in Surfactant Research. Karger, Basel München Paris pp 93-92
11. Bhattacharyya SN, Lynn WS (1975) Isolation and characterization of a hydroxyproline containing glycoprotein from normal animal lung lavage and lamellar bodies. J Clin Invest 55:914-920
12. Bhattacharyya SN, Sahu S, Lynn WS (1976) Structural studies on a glycoprotein isolated from alveoli of patients with alveolar proteinosis. Biochim Biophys Acta 427:91-106
13. Bignon J, Chahinian P, Feldmann G, Sapin C (1975) Ultrastructural immunoperoxidase demonstration of autologous plasma proteins to exogenous proteinic tracers. Proc Soc Exptl Biol Med 109:369-371
14. Bignon J, Jaubert F, Jaurand MC (1977) Ultrastructural study of a glycoprotein isolated from alveoli of patients with alveolar proteinosis. Biochim Biophys Acta 537:329-335
15. Bignon J, Chahinian P, Feldmann G, Sapin C (1975) Ultrastructural immunoperoxidase demonstration of autologous albumin in the alveolar capillary membrane and in the alveolar lining material in normal rats. J Cell Biol 64:503-504
16. Bignon J, Jaurand MC, Pinchon MC, Sapin C, Warnet JM (1976) Immunoelectron microscopic and immunochromatographic demonstration of serum proteins in the alveolar lining material of the rat lung. Am Rev Respir Dis 113:109-120
17. Bignon J, Jaubert F, Jaurand MC (1977) Ultrastructural basis of the pulmonary capillary permeability to autologous plasma proteins to exogenous proteinic tracers. Chest 71 (Suppl): 294-296
18. Brown ES (1957) Lung area from surface tension effects. Proc Soc Exptl Biol Med 95:168-170
19. Brown ES (1964) Isolation and assay of dipatmitoyl lecithin in lung extracts. J Am Physiol 207:402-406
20. Claypool WD, Chander A, Fisher AB (1981) Isolation of the hydrophobic apoproteins of rat lung surfactant. Fed Proc 40:408
21. Clements JA (1957) Surface tension of lung extracts. Exptl Biol Med 95:170-172
22. Clements JA, Brown ES, Johnson RP (1958) Pulmonary surface tension and the mucus lining of the lungs. Some theoretical considerations. J Appl Physiol 8:191-203
23. Clements JA, King RJ (1976) Composition of the surface active material. In: Crystal RG (Ed) Lung Biology in Health and Disease, Vol 2. Marcel Dekker, New York, pp 363-387
24. Cochrane CG, Spragg P, Revak SA (1983) Pathogenesis in adult respiratory distress syndrome. J Clin Invest 71:754-761
25. Gadek JE, Fields KA, Zimmermann RL, Rennard SJ, Crystal RG (1981) Antielastases of the human alveolar structure. Implications for the protease-antiprotease theory of emphysema. J Clin Invest 68:889-898
26. George G, Hook GER (1984) The pulmonary extracellular lining. Environ Health Perspect 55:227-237
27. van Golde LMG (1976) Metabolism of phospholipids in the lung. Am Rev Respir Dis 107:784-789
28. Gotto R, Ueda S, Nakayama Y, Takashita Y, Yasuoka S, Tsukura E (1983) Protein components of bronchoalveolar lavage fluids from non-smokers and smokers. Eur J Respir Dis 64:369-377
29. Gruenwald P, Johnson RP, Hunstead RJ, Clements JA (1962) Correlation of mechanical properties of infant lungs with surface activity of extracts. Proc Soc Exptl Biol Med 109:369-371
30. Guyton AC, Moffatt DS, Adair TH (1984) Role of alveolar surface tension in transepithelial movement of fluid. In: Robertson B, van Golde LMG, Batenburg JJ (Eds) Pulmonary Surfactant. Elsevier, Amsterdam, pp 171-185
31. Hallmann M, Spragg R, Harrall JH, Moser K, Gluck L (1983) Evidence of lung surfactant abnormality in respiratory failure. J Clin Invest 70:673-683
32. Hand WL, Cantey JR (1974) Antibacterial mechanisms of the lower respiratory tract. Immunoglobulin synthesis and secretion. J Clin Invest 53:354-362
33. Hu PC, Miller FJ, Daniels MJ, Hatch GE, Graham JA, Gardner DE, Selgrade MK (1982) Protein accumulation in lung lavage fluid following ozone exposure. Environ Res 29:377-388
34. Hunninghake GW, Gadek JE, Kawamami O, Ferrans VJ, Crystal RG (1979) Inflammatory and immune processes in the human lung in health and disease: Evaluation by bronchoalveolar lavage. Am J Pathol 97:149-206
35. Janoff A, Carp H, Lee DK, Drew RT (1979) Cigarette smoke inhalation decreases α1-antitrypsin activity in rat lung. Science 206:1313-1314
36. Katyal SL, Reasor MJ (1983) Chlrophomccline-induced alterations in pulmonary phospholipid content in rats. Biochem Pharmacol 32:2683-2688
37. Kaltreider HB (1978) The role of the lung in immunoglobulin metabolism. In: Robin ED (ed) Lung Biology in Health and Disease, Vol 8. Marcel Dekker, New York, pp 431-461
38. Katyal SL, Estes LW, Lombardi B (1979) Method for the isolation of surfactant and lavages of lung of adult, newborn, and fetal rats. Lab Invest 36:585-592
39. Katyal SL, Singh G (1979) An immunologic study of the apoproteins of rat lung surfactant. Lab Invest 40:562-567
40. Katyal SL, Singh G (1981) Analysis of pulmonary surfactant apoproteins by electrophoresis. Biochim Biophys Acta 670:322-331
41. Katyal SL, Amenta JS, Singh G, Silverman JA (1984) Deficient lung surfactant apoproteins in amniotic fluid with mature phospholipid profile from diabetic pregnancies. Am J Obstet Gynaecol 148:48-53
42. King RJ, Clements JA (1972) Surface active materials from dog lung. I. Method of isolation. Am J Physiol 222:707-714
43. King RJ, Clements JA (1972) Surface active materials from dog lung. II. Composition and physiological correlations. Am J Physiol 222:715-726
44. King RJ, Klass DJ, Gikas EG, Clements JA (1973) Isolation of apoproteins from canine surface active material. Am J Physiol 224:788-795
85. Velutti G, Capelli O, Lusuardi M, Braghiroli A, Pellegrino M, Milanti G, Benedetti L (1983) Bronchoalveolar lavage in the normal lung. Respiration 44:403–410
86. Voisin GA (1979) Immune agents of the facilitation reaction. Their possible role in protection of the placental allograft. In: Beconsfield P, Villee C (Eds) Placenta: a neglected experimental animal. Pergamon Press, New York, pp 283–294
87. White R, Janoff A, Godgrey HP (1980) Secretion of α₁-macroglobulin by human alveolar macrophages. Lung 158:9–14
88. Williams MC, Benson BJ (1981) Immunocytochemical localization and identification of the major surfactant protein in adult rat lung. J Histochem Cytochem 29:291–305
89. Zänker KS, Wendt P, Blämel G, Probst J (1980) Partial purification and characterization of phosphatidylcholine-binding proteins from lung lavage. Biochem Med 23:239–256

Received March 22, 1985
Revised June 18, 1985
Accepted June 21, 1985

Prof. Dr. P. von Wichert
Medizinische Poliklinik
Klinikum der Philipps-Universität
Emil-Mannkopff-Straße
D-3550 Marburg