Chapter 25

Surfactant metabolism: factors affecting lipid uptake in vivo and in vitro

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Kurt von Neergaard [1] was the first to draw attention to the role of surface tension within the lung. In 1929, he demonstrated that the pressure needed to inflate a fluid-filled lung with fluid was less than approximately one third to one quarter of the pressure necessary to inflate an air-filled lung with the same volume of air (Fig. 1 [1]). From these experiments he concluded that about two thirds of the retractive forces were due to surface tension phenomena acting at the air–liquid interface within the lung; this implies that this surface tension at the alveolar level is reduced by the presence of a surface-active agent with a low surface tension to allow normal breathing.

Unfortunately, these findings were published only in German, and for approximately 25 years they remained practically unnoticed by other scientists in the

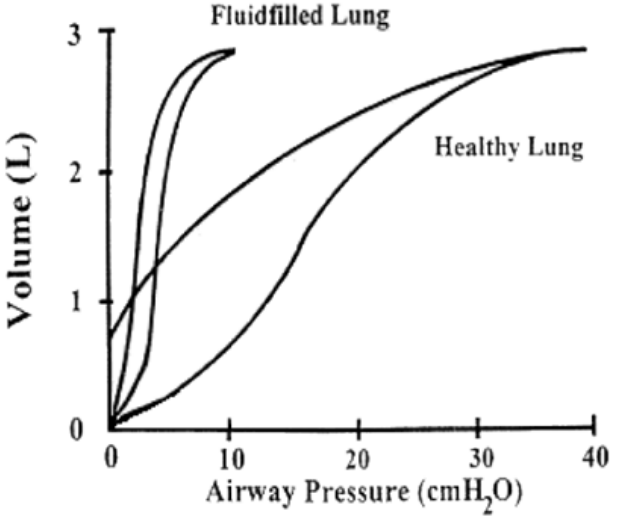

Fig. 1. Pressure volume diagram of a healthy air-filled lung and a lung affected by ARDS. In ARDS higher pressures are required to expand the lung than when the lung is healthy, because of the high surface tension at the air–liquid interface in the alveoli, which is caused by surfactant inactivity. (Adapted from [1])

field. The next description of the presence of a surface active agent within the lung was in 1954 by Macklin, who described a thin aqueous mucoid film that was formed by granular pneumocytes on the alveolar wall and which moved constantly towards bronchioles and phagocytic pneumocytes [2]. The following year, it was noted by Pattle et al. that the foam and bubbles from lung oedema and healthy lung cuts had a remarkable stability; they concluded that these bubbles consisted of a surface-active agent that was able to lower surface tension towards zero [3].

In 1957, Clements used the Wilhelmy balance to demonstrate that the surface tension derived from the alveolar lining fluid of the lung was not a constant value; with a large surface the surface tension was high, but when the surface area was decreased surface tension fell to values near zero [4].

Avery and Mead made the first steps towards extensive research on this surface-active agent, called pulmonary surfactant, by demonstrating higher surface tension in very small premature infants and infants who died of respiratory failure due to hyaline membrane disease [5]. Even today, almost half a century later, small steps are still being made towards better understanding of this surface-active agent.

Pulmonary surfactant

This pulmonary surfactant, lining the alveolar surface, is a complex of lipids and proteins produced in the alveolar type II cells and secreted into the alveolar space.

Lipids

The lipid composition is generally the same in both compartments [6, 7]. Most of the lipids are phospholipids (80–90%), and in decreasing order of content are cholesterol, triacylglycerol and free fatty acids [7] (Fig. 2). Phosphatidylcholine (PC) compromises most of the phospholidids (70–80%), and approximately 50% of it is disaturated (DPPC) [6, 8].

This subgroup of PC is an unusual species, with palmatic acid at both the 1- and the 2-position rather than a saturated fatty acid at the 1-position and an unsaturated fatty acid at the 2-position of the diacylglycerolphospholipid found in most mammalian tissues; although not specific for surfactant (because it is also found in other tissues) it compromises a very high percentage of the surfactant phospholipids.

Even in early fetal gestation, about 20% of the total amount of PC retrieved from the lung is DPPC [9]. This DPPC is the main surface tension-lowering phospholipid in the lung. Although only a small fraction of the extracellular DPPC is necessary to cover the alveolar wall throughout the lung with a monolayer (as calculated by Wright and Clements [10]), its pool size is tightly regulated. For example, short-term decreases in the amount of DPPC due to an abnormal nutritional state, such as fasting [11], fatty acid deficiency [12] or choline deficiency [13], are replenished rapidly by adaptation mechanisms [14].

Next, all mammalian pulmonary surfactants have been shown to contain significant amounts of phosphatidylglycerol (PG) (7–18%) and phosphatidylinositol

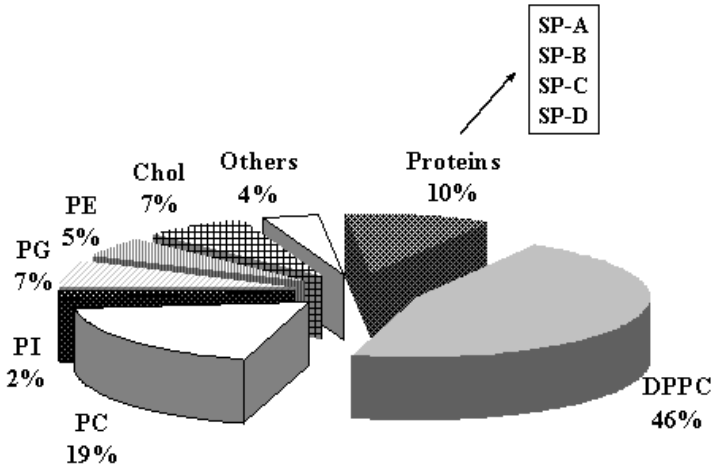

Fig. 2. Schematic overview of surfactant composition. *PC* phosphatidylcholine, *DPPC* dipalmitoylphosphatidylcholine, *PI* phosphatidylinositol, PG phosphatidylglycerol, *PE* phosphatidylethanolamine, *Chol* cholesterol

(PI) (2–4%) [15], suggesting a specific role of these acidic phospholipids. In adult mammalians, PG is the second major lipid component after PC, comprising approximately 5–10% of total surfactant phospholipids in humans [7, 16-18] and rats [6, 19]. However, in preterm fetal lungs the PG component of surfactant is extremely small, although its relative absence is compensated by an increased amount of PI in the surfactant compartments [8, 20–22].

When DPPC is mixed with PG or PI, adsorption of the lipids in the monolayer is enhanced, indicating that these negatively charged lipids may play an important role in the surface tension-lowering activity of surfactant. For PG, this enhanced adsorption may be caused by a specific interaction between PG and SP-B [23, 24].

Finally, the remaining phospholipids consist mainly of phosphatidyl-ethanolamine (PE) (2–3%) and some other minor phospholipids, whereas the total surfactant is completed by cholesterol [15].

Proteins

Pulmonary surfactant contains at least four surfactant proteins (SP), SP-A, SP-B, SP-C and SP-D. Of these proteins, SP-A and SP-D are hydrophilic proteins and SP-B and SP-C are hydrophobic.

SP-A has been studied extensively, and although its role is not yet completely clear it is suggested that it has an important role in regulating surfactant function

via binding to phospholipids [25, 26], modifying phospholipid structure to tubular myelin [27, 28], maintaining the surface properties of surfactant [29], regulating secretion and clearance of surfactant [30-37] and regulating alveolar macrophage function [38], as well having a possible role in the immunological properties of surfactant [38, 39].

SP-B and SP-C are two hydrophobic proteins that are known to play an important part in the formation of a stable lipid monolayer. Especially SP-B has been shown to be essential for normal surfactant function, lowering surface tension [40, 41]; absence of SP-B at birth leads to death caused by respiratory insufficiency [42, 43], and conditional knockout of SP-B in adult animals leads to respiratory failure [44]. In addition, it has been suggested that SP-B has a role in protection of the surfactant system against endotoxin-induced lung inflammation by enhancing surfactant function, resulting in reduced lung injury, decreased influx of inflammatory cells and lower cytokine levels [45].

SP-C also enhances the surface-active properties of surfactant [40, 41, 46-48]. Although (unlike SP-B) its absence at birth is not lethal, it does result in decreased stability of surfactant at low volumes even though surfactant pool sizes and lung morphology are similar in wild-type and SP-C knockout mice [49]. Another function of SP-C is to increase the resistance of surfactant against inactivation by plasma proteins [50]. On the other hand, elevated expression of SP-C is thought to be related to cytotoxicity and, ultimately, altered lung development [51]. Though it is thought that SP-D might be the fourth surfactant protein, it is not found only within the lung but also in other organ systems, and its specific contribution with regard to surfactant is not completely clear; however, several studies have suggested that, together with SP-A, it has an immunomodulatory role in the lung [39, 52-55].

Metabolism

The presence of surfactant within the alveolus is the result of a complex system of production, secretion, insertion into the lipid monolayer and turnover, uptake and recycling (Fig. 3).

Production and secretion

Surfactant phospholipids are produced by alveolar type II cells which comprise only 15% of the total number of cells in the lung [56-58]. The de novo synthesis of surfactant is thought to be relatively slow, especially in newborn animals [59, 60] and also in humans, as demonstrated by Bunt et al. using stable isotopes [61]. Bunt et al. also demonstrated that the use of prenatal corticosteroids increased surfactant synthesis in the preterm infant [62] and in very premature baboons [63]. Therefore, most surface-active surfactant is produced by recycling. Martini et al. have demonstrated that approximately 50–90% of the PC in surfactant is recycled, depending on age and species, the contribution of recycling decreasin with increasing age [64]. The surfactant lipids are synthesised in the endoplasmatic reticulum and then stored

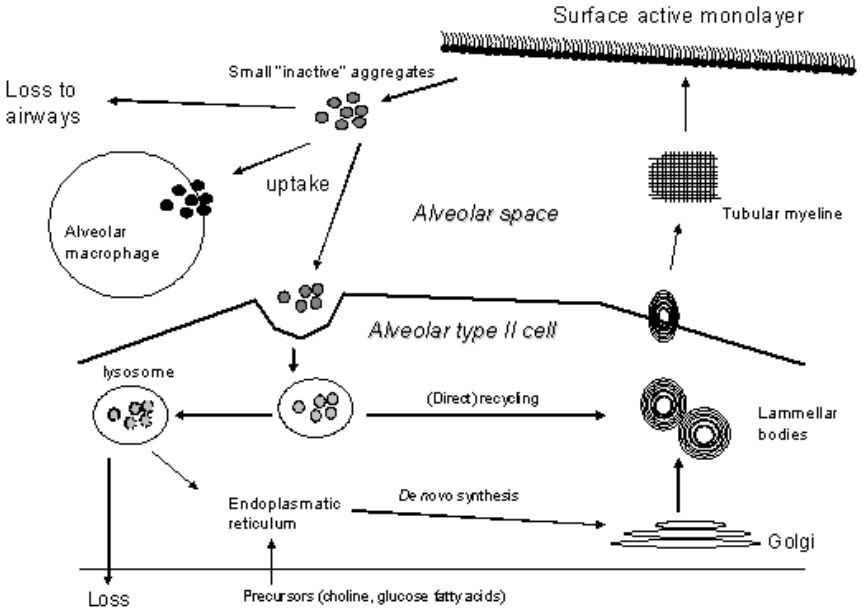

Fig. 3. Diagram showing surfactant metabolism

in lamellar bodies [65, 66]. In these lamellar bodies surfactant-specific proteins A, B and C are already present [67]; however, the content of SP-A is extremely low (1%), suggesting that the SP-A present in the alveolar space might be derived from parts other than lamellar bodies. When the alveolar type II cell is stimulated, intracellular effectors diffuse and activate the movement of the lamellar bodies to the apical plasma membrane of the alveolar type II cells and the content of the lamellar bodies is secreted into the alveolar space by a process of regulated exocytosis [58, 68, 69]. (For more details on the regulation of secretion see [56, 70, 71].)

After excretion into the alveolar space the lamellar bodies unravel and form tubular myelin after association with SP-A [28, 72]. Subsequently, the material of the lamellar bodies is absorbed/inserted into the lipid monolayer. This tubular myelin is most probably the immediate precursor for lipids inserted into the monolayer; however, it should be noted that SP-A knockout mice do not produce normal tubular myelin and the structure and in vitro properties of surfactant have changed. However, the in vivo function of surfactant in SP-A knockout mice has not changed, and thus tubular myelin is not essential for normal lung function [74].

Conversion of surfactant

During respiration the surfactant in the lipid monolayer is converted from large surface-active aggregates into small inactive surfactant aggregates [74–76]. However, little is known about the exact mechanism of this conversion. These small aggregates are not surface active and are removed from the alveolar space to be reutilised or recycled to ensure the presence of surface active aggregates in the lipid monolayer.

Uptake/removal of small aggregates

The converted, inactivated surfactant is cleared from the alveolar space mainly by way of uptake by alveolar type II cells and alveolar macrophages. However, their relative contribution to the uptake of surfactant lipids remains obscure and is dependent on several factors. In vitro studies suggest a major role for alveolar macrophages [77], whereas in vivo experiments suggest an equal contribution or even a major role for alveolar type II cells in the clearance of surfactant [78–80].

Because of the need for recycling, as suggested previously, re-uptake of surfactant by alveolar type II cells is essential, and this is possibly a crucial factor in the surfactant metabolism. Unfortunately, little is known about the regulation and mechanisms of removal of surfactant from the alveolar space by alveolar type II cells and alveolar macrophages.

This uptake of surfactant lipids is thought to take place (at least in part) via a coated-pit pathway [81–83]. More recently, it was demonstrated that all surfactant phospholipids are internalised via the same pathway by alveolar macrophages and alveolar type II cells, though alveolar cells have a higher affinity for negatively charged phospholipids [84]. In addition, the surfactant proteins are known to affect the uptake, especially SP-A [34, 36, 80, 85, 86].

Measuring uptake

Most studies on surfactant metabolism, especially those focused on the uptake of surfactant lipids by alveolar type II cells and alveolar macrophages, have used radioactive labeled DPPC to measure the uptake. In addition, most studies have been performed in an in vitro setting, whereas in vivo studies have focused mainly on alveolar macrophages. Unfortunately, the use of radioactivity does not discriminate between uptake or intracellular presence of the label and association with the outer cell membrane or adherence. In addition, it is not possible to specify precisely which cells are involved in the 'uptake', as whole-lung tissue is tested for radioactivity.

Recently, we described a method using fluorescence-labeled liposomes to study the uptake of surfactant-like liposomes both in vivo and in vitro and in both alveolar type II cells and alveolar macrophages [87]. Our method mimics the small aggregates of surfactant, as these are the surfactant aggregates generally thought to

be removed from the alveolar space. In addition, confocal laser microscopy can be used to demonstrate that the fluorescence-labeled liposomes are indeed intracellularly located rather than adherent to the outer cell membrane.

More interestingly, when our method is used it is not DPPC that is labeled but PE, a minor component of surfactant, as a part of liposomes consisting of the main lipid components of surfactant, providing the opportunity to study the role of the main lipid components in regulation of the uptake of surfactant.

One of the advantages of the use of fluorescence-labeled liposomes is the possibility of focusing on one particular cell type; with the use of specific fluorescence-labeled antibodies it is possible to discriminate between different cell types and study their relative contributions or roles in the removal of surfactant lipids and possible mutual regulation of the uptake; even more specifically, it is possible to determine whether all cells or just a subpopulation of cells are involved in the uptake. Another important advantage of the method described by our group is that it allows the measurement of uptake by alveolar type II cells and alveolar macrophages both in vivo and in vitro. We believe we were the first to demonstrate that in vivo a significantly smaller percentage of the alveolar type II cells is involved in the uptake than in vitro; this indicates the need to study uptake both in vitro and in vivo (Fig. 4).

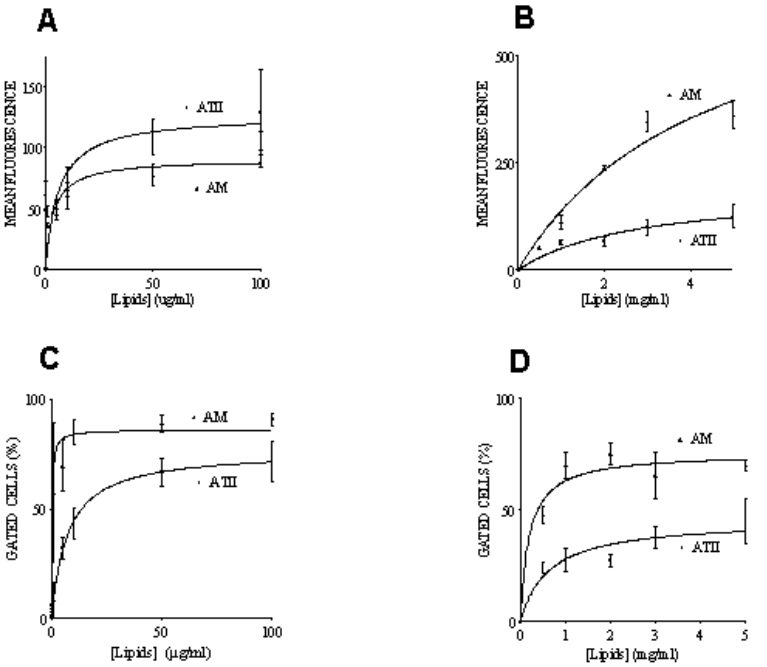

Fig. 4A–D. Differences in uptake in vivo and in vitro. Cell-associated fluorescence as a measure of the uptake of surfactant-like liposomes was determined for different concentrations of labeled liposomes both in vitro (**A**, **C**) and in vivo (**B**, **D**). In addition, the percentage of cells involved in the uptake was determined. (Data derived from [94]). *ATII* alveolar type II cells, *AM* alveolar macrophages

Effect of lipid composition on uptake

The composition of surfactant is largely similar across different species, including humans [88, 89]; however, small differences in the relative concentrations of the individual lipids are observed, which are also related to age. For example, fetal or neonatal surfactant contains a larger percentage of phosphatidylinositol and less phosphatidylglycerol, whereas adult surfactant contains more phosphatidylglycerol than phosphatidylinositol [15]. In addition, neonates have been shown to rely more on recycling, and thus uptake, than do adults [59, 64], which suggests a possible effect/role of the lipid composition on the uptake. Moreover, severe lung injury (initiated by a wide variety of causes) is known to be related to alterations in the lipid composition of surfactant [90, 91], which could also contribute to a decreased surfactant function, implying an effect of the different surfactant lipids on the uptake. However, it is still not known how these alterations in composition are related to the disease.

Bates et al. [92] and Chander et al. [93] were the first to report on the influence of the individual surfactant lipids on uptake, demonstrating that radiolabeled PG was cleared more rapidly by alveolar type II cells in vitro. This higher uptake of PG than of DPPC in vitro was also demonstrated for alveolar macrophages [94].

More recently, we have demonstrated a common pathway for the uptake of surfactant lipids by both cell types in vitro [84]. A significantly lower percentage of alveolar type II cells than of alveolar macrophages is involved in the uptake of DPPC (29% vs 72%, respectively), whereas the number of cells involved in the uptake of PG is approximately the same. The presence of a possible phospholipid receptor would simplify the explanation of these results. A different distribution of this phospholipid receptor to alveolar macrophages and type II cells might be the reason for the difference in the percentages of these cells involved in the uptake. The uptake of DPPC requires more receptors than the uptake of, for instance, PG; or, more generally, more negatively charged than neutrally charged phospholipids are taken up, and the negatively charged phospholipids are taken up more easily. A higher receptor density on alveolar macrophages than on alveolar type II cells, and the presence of several subpopulations of type II cells with different receptor densities could explain the lower percentage of alveolar type II cells than of alveolar macrophages.

These results relating to the role of individual surfactant lipids indicate that, besides surfactant proteins, the phospholipid composition of the small aggregates affects the surfactant metabolism. However, the relevant studies were performed in vitro, whereas significant differences have been demonstrated in the uptake of surfactant-like liposomes by alveolar cells in in vivo and in vitro experiments, and extrapolation of these results to the in vivo situation should be done with caution [87].

The effects of lipid composition in vivo was studied by increasing the amount of PG, the second major phospholipid, present in the small aggregates. The incorporation of PG influences the uptake of surfactant-like liposomes by alveolar cells, though the effects on the two cell types differ. The uptake of surfactant-like liposomes by alveolar type II cells is hardly affected by different concentrations of

PG. More interestingly, however, the influence of the intratracheal instillation of PG-containing liposomes on alveolar macrophages is dramatic; in particular, the number of alveolar macrophages obtained in the lung lavage is influenced by the amount of PG. In addition, not only does an increase in the amount of PG reduce the number of alveolar macrophages, but this decrease in the number of cells is accompanied by a deterioration in arterial oxygenation. Although PG does not interfere with the function of endogenous surfactant in vitro, as was tested, its increase does lead to reduced surface activity in vivo. Moreover, these adverse affects of PG on endogenous surfactant function can be avoided by adding so-called co-factors, such as calcium or magnesium.

The 'fatal' effect of PG on alveolar macrophages, as suggested by our group, is absent in vitro, because in that setting the aforementioned co-factors are already present during incubation. However, the effects of PG on the uptake of surfactant-like liposomes by alveolar type II cells in vivo are completely different from those derived from the in vitro experiments, even when co-factors are present. In vitro, increased concentrations of PG result in an increased uptake of these liposomes by alveolar type II cells, whereas the uptake of these liposomes by alveolar type II cells in vivo is hardly affected by the concentration of PG within the liposomes, irrespective of the presence of the suggested co-factors. These results underline the presence of 'environmental' factors that influence the uptake in vivo and thus emphasise the need to study the uptake of lipids and/or surfactant by alveolar type II cells and alveolar macrophages both in vivo and in vitro.

Effect of surfactant proteins

As previously mentioned, surfactant contains four proteins: SP-A, SP-B, SP-C and SP-D. The first, SP-A, has been extensively studied and is thought to fulfil several roles within surfactant homeostasis, especially in regulation of the clearance of surfactant from the alveolar space [85].

Next, both SP-B and SP-C are known to be important for the surface-active surfactant monolayer [40–44, 95, 96]. On the other hand, as far as the effects on the uptake of surfactant by alveolar cells are concerned, SP-B is capable of increasing the uptake of lipids by alveolar cells [36, 97] (Poelma et al., submitted for publication); however, high concentrations of SP-B are required to induce this increase, which raises the question of the physiological contribution of SP-B to regulation of the uptake of surfactant lipids by alveolar cells. On the other hand, SP-C has a similar function to SP-B with regard to enhancing surface-active properties of surfactant [40, 41, 46–48], but it should be noted that SP-C increases the uptake of surfactant-like liposomes at lower concentrations than SP-B. This effect of SP-C is concentration dependent, with a maximum at 2% SP-C. In addition, the presence of co-factors (such as calcium) within the liposomes decreasing the possibility of dilution of the endogenous pool has been shown to further increase the effect of SP-C. When 1% SP-C is incorporated the uptake is already increased, but the maximum increase is at 2% (Poelma et al., submitted for publication). However, the effects of SP-C on the uptake of surfactant-like liposomes are suggested to be

suppressed in vivo, since in vitro experiments have shown a much larger effect, more specifically a nonsaturable effect, on uptake [36, 97]. Furthermore, SP-C is known to combine very rapidly with lung tissue and alveolar macrophages [98, 99]. This increased association, coming about even more rapidly than an association with DPPC, might be an explanation for the increased uptake of liposomes containing SP-C. Nonetheless, other factors, such as the conformational changes observed in liposomes after the incorporation of SP-C, may also affect the binding and uptake of these liposomes by alveolar cells, as suggested by Rice et al. [100]. Finally, the presence of a putative SP-C receptor could also induce increased uptake. However, because its presence has not yet been demonstrated, further studies are needed on this point.

Effect of surfactant therapy

Currently, exogenous surfactant is increasingly used in the clinical setting, mostly in neonates but its use in adults is now under consideration [101]. However, the administration of exogenous surfactant is known to influence the endogenous surfactant. Most studies have focused on the effects of exogenous surfactant on the production and/or secretion of DPPC, and they have yielded conflicting results [33, 102–105]. In premature infants with respiratory distress syndrome, treatment with exogenous surfactant stimulates the synthesis of endogenous surfactant [106]. Little is known about the clearance or uptake of surfactant. Exogenous surfactant is taken up by alveolar type II cells and alveolar macrophages [107–10]; however, the specific effects of exogenous surfactant, i.e. surface-active surfactant, on the clearance of non-surface-active surfactant, whether endogenous or exogenous, is unknown. Our group has demonstrated significant effects of exogenous surfactant on the clearance of surfactant-like liposomes (unpublished data). Nevertheless, the effect of exogenous surfactant on uptake differs significantly between in vivo and in vitro conditions.

Effect of surfactant protein analogues

As previously mentioned, SP-B is essential for the biophysical properties of pulmonary surfactant, and its presence is thus the most highly appreciated in exogenous surfactant.

The high cost of naturally derived exogenous surfactant increases the demand for a synthetically produced surfactant. Therefore, synthetic analogues of SP-B based on the known human amino-acid sequence have been tested and closely mimic the function of natural surfactant proteins [111]. In addition, these SP-B analogues might be optimised: only essential parts of SP-B are reproduced and further developed, to increase the efficiency of SP-B within the exogenous surfactant preparation. SP-B analogues are based on the 1-25 sequence of the N-terminal site of human SP-B with a modification at position 11: cysteine is replaced by alanine (Cys-11>Ala-11) [112, 113]. A mutant SP-B (serine SP-B-1-25) was synthesised with site-specific substitu-

tion of serine for arginine in positions 12 and 17 and for lysine in positions 16 and 24 of the N-terminal (Fig. 5). A disulfide-linked homodimer of these SP-B analogues was formed by oxidising the monomeric SP-1-25 peptide [112, 113].

The serine-SP-B-1-25 analogues have been shown to be less surface active than the SP-B-1–25 variants. We have shown that the less surface-active SP-B analogues, the SP-B serine variations, reduce the uptake of surfactant-like liposomes by alveolar type II cells when incorporated into the liposomes; on the other hand, the SP-B-1-25 analogues mimic the effect of native SP-B and do not induce any changes in the uptake of liposomes by alveolar type II cells (unpublished data). With regard to the uptake of these liposomes with SP-B analogues incorporated by alveolar macrophages, our group has demonstrated that the surface-active SP-B analogues influence the uptake by alveolar macrophages (unpublished data).

SP-C has also been shown to enhance the surface-tension-lowering properties of surfactant; therefore, surfactant preparations intended for clinical use will most probably contain not only SP-B but also SP-C. The use of recombinant SP-C (rSP-C) in surfactant preparations is under investigation, to establish the efficiency of this SP-C in enhancing the surface-tension-lowering activities of surfactant [111, 114–116]. In terms of surface-tension-lowering activity, rSP-C surfactant (Altana, Konstanz, Germany) has similar results to natural surfactant [117, 118]. This rSP-C surfactant contains an SP-C that is an analogue of human SP-C; it contains pheny-

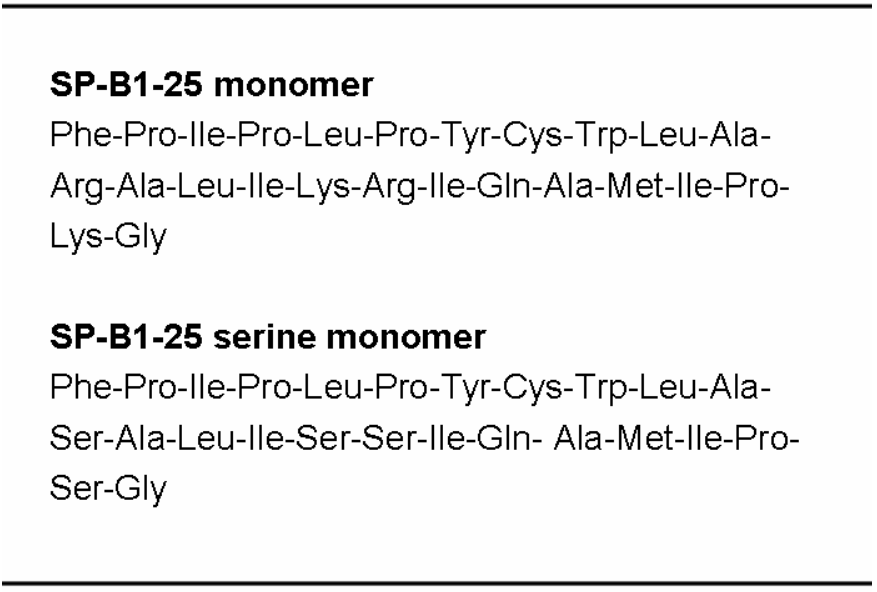

Fig. 5. Peptide sequences of the SP-B analogues and their serine mutants. The SP-B1-25 homodimer consists of two SP-B1-25 monomers disulfide-linked at Cys8 (not shown). The fluorescent label was inserted in all peptides at the N-terminus, shown at the left side of the sequence

lalanine instead of two cysteines in positions 4 and 5 of the human SP-C sequence, and isoleucine instead of methionine in position 32. However, the effects of these SP-C-analogues on the uptake, or more generally on the metabolism of surfactant, are not known. Our group has shown that the uptake of surfactant lipids by alveolar type II cells and alveolar macrophages is regulated by SP-C (Poelma et al., submitted for publication), and the influence of recombinant SP-C on surfactant metabolism therefore needs to be clarified.

Additional factors influencing surfactant uptake

Finally, besides factors related directly to surfactant, our group has shown that multiple 'environmental' factors influence and affect the surfactant metabolism. Some of these factors have been described previously; for example, calcium has been shown to influence the metabolism, insofar as its presence PG promotes the association of SP-A and DPPC [119, 120], and to affect the function of SP-B [29, 121]. The effects or influences of these alveolar factors (e.g. divalent cations, as suggested by the study of our group with regard to the effects of SP-B and SP-C) are also underlined by the fact that in vivo and in vitro results differ significantly, even when the absence or dilution of known co-factors such as calcium are compensated for. It should be emphasised that in our opinion in vitro experiments are indeed useful, although caution must be exercised in extrapolation of their results to the in vivo situation. Use of a similar technique for both in vivo and in vitro studies enables the researcher to compare the results and might help to clarify the complex mechanism of the regulation of uptake of surfactant lipids by alveolar cells in vivo.

In addition, although most studies on the uptake of surfactant have focused on healthy animals, many different diseases can disturb the surfactant system, and the presence of cytokines and other inflammatory parameters are known to affect the presence of surface-active surfactant in the lung. For example, tumor necrosis factor (TNF)-α, interleukin (IL) -1 and interferon (IFN)-γ are known to influence the production of SP-A, SP-B and SP-C, which regulates the uptake of surfactant by alveolar cells and thus affects the total metabolism [122–126]. In addition, prenatal steroids have been shown to increase surfactant synthesis [62].

Future studies

Because the uptake of surfactant in healthy adult animals has been clarified to some extent, future research could focus on the uptake of surfactant-like liposomes, with different models used for diseased animals. Possible irregularities in uptake and thus in endogenous surfactant metabolism might be elucidated. The known regulatory factors, at least those clarified hitherto, will then provide options for restoration of normal metabolism by influencing the uptake. For example, if uptake of surfactant is reduced in a certain disease state, it might be beneficial to increase the concentration of PG within the surfactant preparation used for therapy. In other words, clarifying regulating factors in the surfactant uptake and uncovering irregularities in the meta-

bolism, or more specifically in the uptake of surfactant, will allow the development of an exogenous surfactant preparation that is disease specific, by modifying the composition depending on the underlying deviation from normal.

References

1. Von Neergaard K (1929) Neue Auffassungen über einen Grundbegriff der Atemmechanik; Die Retraktionskraft der Lunge, abhängig von deer Oberflächenspannung in den Alveolen. Z Ges Exp Med 66:373-394
2. Macklin CC (1954) The pulmonary alveolar mucoid film and pneumocytes. Lancet II:1099-1104
3. Pattle RE (1955) Properties, function, and origin of the alveolar lining layer. Nature 175:1125-1126
4. Clements JA (1957) Surface tension of lung extracts. Proc Soc Exp Biol Med 95:170-172
5. Avery ME, Mead J (1959) Surface properties in relation to atelectasis and hyaline membrane disease. Am J Dis Child 97:517-523
6. Adachi H, Hayashi H, Sato H et al (1989) Characterization of phospholipids accumulated in pulmonary-surfactant compartments of rats intratracheally exposed to silica. Biochem J 262:781-786
7. King RJ (1984) Isolation and chemical composition of pulmonary surfactant. In: Robertson B, Van Golde LM, Batenburg JJ (eds) Pulmonary surfactant: from molecular biology to clinical practice. Elsevier Science, Amsterdam, pp 1-15
8. Hayashi H, Adachi H, Kataoka K et al (1990) Molecular species profiles of acidic phospholipids in lung fractions of adult and perinatal rabbits. Biochim Biophys Acta 1042:126-131
9. Okano G, Akino T (1978) Changes in the structural and metabolic heterogeneity of phosphatidylcholines in the developing rat lung. Biochim Biophys Acta 528:373-384
10. Wright JR, Clements JA (1987) Metabolism and turnover of lung surfactant. Am Rev Respir Dis 136:426-444
11. Gail DB, Massaro GD, Massaro D (1977) Influence of fasting on the lung. J Appl Physiol 42:88-92
12. Nakamura M, Kawamoto T, Akino T (1980) Dietary regulation of dipalmitoyl phosphatidylcholine in the lung. Effects of essential fatty acid deficiency. Biochim Biophys Acta 620:24-36
13. McMahon KE, Farrell PM (1986) Effect of choline deficiency on lung phospholipid concentrations in the rat. J Nutr 116:936-943
14. Brown LA, Bliss AS, Longmore WJ (1984) Effect of nutritional status on the lung surfactant system: food deprivation and caloric restriction. Exp Lung Res 6:133-147
15. Veldhuizen R, Nag K, Orgeig S et al (1998) The role of lipids in pulmonary surfactant. Biochim Biophys Acta 1408:90-108
16. Honda Y, Tsunematsu K, Suzuki A et al (1988) Changes in phospholipids in bronchoalveolar lavage fluid of patients with interstitial lung diseases. Lung 166:293-301
17. Hallman M, Spragg R, Harrell JH et al (1982) Evidence of lung surfactant abnormality in respiratory failure. Study of bronchoalveolar lavage phospholipids, surface activity, phospholipase activity, and plasma myoinositol. J Clin Invest 70:673-683
18. Robinson PC, Watters LC, King TE et al (1988) Idiopathic pulmonary fibrosis. Abnormalities in bronchoalveolar lavage fluid phospholipids. Am Rev Respir Dis 137:585-591

19. Hallman M, Gluck L (1975) Phosphatidylglycerol in lung surfactant. II. Subcellular distribution and mechanism of biosynthesis in vitro. Biochim Biophys Acta 409:172-191
20. Hallman M, Gluck L (1980) Formation of acidic phospholipids in rabbit lung during perinatal development. Pediatr Res 14:1250-1259
21. Benson BJ, Kitterman JA, Clements JA et al (1983) Changes in phospholipid composition of lung surfactant during development in the fetal lamb. Biochim Biophys Acta 753:83-88
22. Hallman M, Feldman BH, Kirkpatrick E et al (1977) Absence of phosphatidylglycerol (PG) in respiratory distress syndrome in the newborn. Study of the minor surfactant phospholipids in newborns. Pediatr Res 11:714-720
23. Baatz JE, Elledge B, Whitsett JA (1990) Surfactant protein SP-B induces ordering at the surface of model membrane bilayers. Biochemistry 29:6714-6720
24. Yu SH, Possmayer F (1990) Role of bovine pulmonary surfactant-associated proteins in the surface-active property of phospholipid mixtures. Biochim Biophys Acta 1046:233-241
25. King RJ, Phillips MC, Horowitz PM et al (1986) Interaction between the 35 kDa apolipoprotein of pulmonary surfactant and saturated phosphatidylcholines. Effects of temperature. Biochim Biophys Acta 879:1-13
26. Kuroki Y, Akino T (1991) Pulmonary surfactant protein A (SP-A) specifically binds dipalmitoylphosphatidylcholine. J Biol Chem 266:3068-3073
27. Williams MC, Hawgood S, Hamilton RL (1991) Changes in lipid structure produced by surfactant proteins SP-A, SP-B, and SP-C. Am J Respir Cell Mol Biol 5:41-50
28. Suzuki Y, Fujita Y and Kogishi K (1989) Reconstitution of tubular myelin from synthetic lipids and proteins associated with pig pulmonary surfactant. Am Rev Respir Dis 140:75-81
29. Hawgood S, Benson BJ, Schilling J et al (1987) Nucleotide and amino acid sequences of pulmonary surfactant protein SP 18 and evidence for cooperation between SP 18 and SP 28-36 in surfactant lipid adsorption. Proc Natl Acad Sci USA 84:66-70
30. Wirtz HR, Dobbs LG (1990) Calcium mobilization and exocytosis after one mechanical stretch of lung epithelial cells. Science 250:1266-1269
31. Rice WR, Ross GF, Singleton FM et al (1987) Surfactant-associated protein inhibits phospholipid secretion from type II cells. J Appl Physiol 63:692-698
32. Kuroki Y, Mason RJ, Voelker DR (1988) Pulmonary surfactant apoprotein A structure and modulation of surfactant secretion by rat alveolar type II cells. J Biol Chem 263:3388-3394
33. Dobbs LG, Wright JR, Hawgood S et al (1987) Pulmonary surfactant and its components inhibit secretion of phosphatidylcholine from cultured rat alveolar type II cells. Proc Natl Acad Sci USA 84:1010-1014
34. Bates SR, Dodia C, Fisher AB (1994) Surfactant protein A regulates uptake of pulmonary surfactant by lung type II cells on microporous membranes. Am J Physiol 267:L753-L760
35. Horowitz AD, Kurak K, Moussavian B et al (1997) Preferential uptake of small-aggregate fraction of pulmonary surfactant in vitro. Am J Physiol 273:L468-L477
36. Horowitz AD, Moussavian B, Whitsett JA (1996) Roles of SP-A, SP-B, and SP-C in modulation of lipid uptake by pulmonary epithelial cells in vitro. Am J Physiol 270:L69-L79
37. Tsuzuki A, Kuroki Y, Akino T (1993) Pulmonary surfactant protein A-mediated uptake of phosphatidylcholine by alveolar type II cells. Am J Physiol 265:L193-L199
38. van Iwaarden JF (1992) Surfactant and the pulmonary defense system. In: Robertson B, van Golde LMG, Batenburg JJ (eds) Pulmonary surfactant: from molecular biology to clinical pratice. Elsevier, Amsterdam, pp 215-228
39. Kramer BW, Speer CP (2003) [Surfactant proteins A and D: major factors of the immune response of the lung]. Z Geburtshilfe Neonatol 207:41-47

40. Curstedt T, Jornvall H, Robertson B et al (1987) Two hydrophobic low-molecular-mass protein fractions of pulmonary surfactant. Characterization and biophysical activity. Eur J Biochem 168:255-262
41. Revak SD, Merritt TA, Degryse E et al (1988) Use of human surfactant low molecular weight apoproteins in the reconstitution of surfactant biologic activity. J Clin Invest 81:826-833
42. Stahlman MT, Gray MP, Falconieri MW et al (2000) Lamellar body formation in normal and surfactant protein B-deficient fetal mice. Lab Invest 80:395-403
43. Tokieda K, Whitsett JA, Clark JC et al (1997) Pulmonary dysfunction in neonatal SP-B-deficient mice. Am J Physiol 273:L875-882
44. Melton KR, Nesslein LL, Ikegami M et al (2003) SP-B deficiency causes respiratory failure in adult mice. Am J Physiol Lung Cell Mol Physiol 285:L543-L549
45. Epaud R, Ikegami M, Whitsett JA et al (2003) Surfactant protein B inhibits endotoxin-induced lung inflammation. Am J Respir Cell Mol Biol 28:373-378
46. Notter RH, Shapiro DL, Ohning B et al (1987) Biophysical activity of synthetic phospholipids combined with purified lung surfactant 6000 dalton apoprotein. Chem Phys Lipids 44:1-17
47. Warr RG, Hawgood S, Buckley DI et al (1987) Low molecular weight human pulmonary surfactant protein (SP5): isolation, characterization, and cDNA and amino acid sequences. Proc Natl Acad Sci USA 84:7915-7919
48. Takahashi A, Fujiwara T (1986) Proteolipid in bovine lung surfactant: its role in surfactant function. Biochem Biophys Res Commun 135:527-532
49. Glasser SW, Burhans MS, Korfhagen TR et al (2001) Altered stability of pulmonary surfactant in SP-C-deficient mice. Proc Natl Acad Sci U S A 98:6366-6371
50. Venkitaraman AR, Hall SB, Whitsett JA et al (1990) Enhancement of biophysical activity of lung surfactant extracts and phospholipid-apoprotein mixtures by surfactant protein A. Chem Phys Lipids 56:185-194
51. Conkright JJ, Na CL, Weaver TE (2002) Overexpression of surfactant protein-C mature peptide causes neonatal lethality in transgenic mice. Am J Respir Cell Mol Biol 26:85-90
52. Kishor U, Madan T, Sarma PU et al (2002) Protective roles of pulmonary surfactant proteins, SP-A and SP-D, against lung allergy and infection caused by Aspergillus fumigatus. Immunobiology 205:610-618
53. Griese M (2002) Respiratory syncytial virus and pulmonary surfactant. Viral Immunol 15:357-363
54. McCormack FX, Whitsett JA (2002) The pulmonary collectins, SP-A and SP-D, orchestrate innate immunity in the lung. J Clin Invest 109:707-712
55. Crouch EC (2000) Surfactant protein-D and pulmonary host defense. Respir Res 1:93-108
56. Rooney SA, Young SL, Mendelson CR (1994) Molecular and cellular processing of lung surfactant. FASEB J 8:957-967
57. Van Golde LM, Batenburg JJ, Robertson B (1988) The pulmonary surfactant system: biochemical aspects and functional significance. Physiol Rev 68:374-455
58. Wright JR, Dobbs LG (1991) Regulation of pulmonary surfactant secretion and clearance. Annu Rev Physiol 53:395-414
59. Jacobs H, Jobe A, Ikegami M et al (1982) Surfactant phosphatidylcholine source, fluxes, and turnover times in 3-day-old, 10-day-old, and adult rabbits. J Biol Chem 257:1805-1810
60. Jobe A, Ikegami M, Sarton-Miller I et al (1980) Surfactant metabolism of newborn lamb lungs studied in vivo. J Appl Physiol 49:1091-1098
61. Bunt JE, Zimmermann LJ, Wattimena JL et al (1998) Endogenous surfactant turnover in preterm infants measured with stable isotopes. Am J Respir Crit Care Med 157:810-814

62. Bunt JE, Carnielli VP, Darcos Wattimena JL et al (2000) The effect in premature infants of prenatal corticosteroids on endogenous surfactant synthesis as measured with stable isotopes. Am J Respir Crit Care Med 162:844-849
63. Bunt JE, Carnielli VP, Seidner SR et al (1999) Metabolism of endogenous surfactant in premature baboons and effect of prenatal corticosteroids. Am J Respir Crit Care Med 160:1481-1485
64. Martini WZ, Chinkes DL, Barrow RE et al (1999) Lung surfactant kinetics in conscious pigs. Am J Physiol 277:E187-E195
65. Askin FB and Kuhn C (1971) The cellular origin of pulmonary surfactant. Lab Invest 25:260-268
66. Chevalier G, Collet AJ (1972) In vivo incorporation of choline- 3 H, leucine- 3 H and galactose- 3 H in alveolar type II pneumocytes in relation to surfactant synthesis. A quantitative radoautographic study in mouse by electron microscopy. Anat Rec 174:289-310
67. Oosterlaken-Dijksterhuis MA, van Eijk M, van Buel BL et al (1991) Surfactant protein composition of lamellar bodies isolated from rat lung. Biochem J 274 (Pt 1):115-119
68. Mason RJ and Voelker DR (1998) Regulatory mechanisms of surfactant secretion. Biochim Biophys Acta 1408:226-240
69. Chander A, Fisher AB (1990) Regulation of lung surfactant secretion. Am J Physiol 258:L241-L253
70. Gobran LI, Rooney SA (2004) Pulmonary surfactant secretion in briefly cultured mouse type II cells. Am J Physiol Lung Cell Mol Physiol 286:L331-:L336
71. Rooney SA (2001) Regulation of surfactant secretion. Comp Biochem Physiol A Mol Integr Physiol 129:233-243
72. Poulain FR, Allen L, Williams MC et al (1992) Effects of surfactant apolipoproteins on liposome structure: implications for tubular myelin formation. Am J Physiol Lung Cell Mol Physiol 262:L730-L739
73. Ikegami M, Korfhagen TR, Whitsett JA et al (1998) Characteristics of surfactant from SP-A-deficient mice. Am J Physiol Lung Cell Mol Physiol 275:L247-L254
74. Veldhuizen RA, Marcou J, Yao LJ et al (1996) Alveolar surfactant aggregate conversion in ventilated normal and injured rabbits. Am J Physiol 270:L152-L158
75. Magoon MW, Wright JR, Baritussio A et al (1983) Subfractionation of lung surfactant. Implications for metabolism and surface activity. Biochim Biophys Acta 750:18-31
76. Yamada T, Ikegami M, Jobe AH (1990) Effects of surfactant subfractions on preterm rabbit lung function. Pediatr Res 27:592-598
77. Miles PR, Ma JY, Bowman L (1988) Degradation of pulmonary surfactant disaturated phosphatidylcholines by alveolar macrophages. J Appl Physiol 64:2474-2481
78. Rider ED, Ikegami M, Jobe AH (1990) Intrapulmonary catabolism of surfactant-saturated phosphatidylcholine in rabbits. J Appl Physiol 69:1856-1862
79. Rider ED, Ikegami M, Jobe AH (1992) Localization of alveolar surfactant clearance in rabbit lung cells. Am J Physiol 263:L201-L209
80. Gurel O, Ikegami M, Chroneos ZC et al (2001) Macrophage and type II cell catabolism of SP-A and saturated phosphatidylcholine in mouse lungs. Am J Physiol Lung Cell Mol Physiol 280:L1266-L1272
81. Straubinger RM, Hong K, Friend DS et al (1983) Endocytosis of liposomes and intracellular fate of encapsulated molecules: encounter with a low pH compartment after internalization in coated vesicles. Cell 32:1069-1079
82. Stevens PA, Wissel H, Zastrow S et al (2001) Surfactant protein A and lipid are internalized via the coated-pit pathway by type II pneumocytes. Am J Physiol Lung Cell Mol Physiol 280:L141-L151

83. Muller WJ, Zen K, Fisher AB et al (1995) Pathways for uptake of fluorescently labeled liposomes by alveolar type II cells in culture. Am J Physiol 269:L11-L19
84. Poelma DL, Ju MR, Bakker SC et al (2004) A common pathway for the uptake of surfactant lipids by alveolar cells. Am J Respir Cell Mol Biol 30:751-758
85. Hawgood S (1992) The hydrophilic surfactant protein SP-A: Molecular biology, structure and function. In: Robertson B, van Golde LMG, Batenburg JJ (eds) Pulmonary surfactant: from molecular biology to clinical pratice. Elsevier, Amsterdam, pp 33-54
86. Horowitz AD, Moussavian B, Han ED et al (1997) Distinct effects of SP-A and SP-B on endocytosis of SP-C by pulmonary epithelial cells. Am J Physiol 273:L159-L171
87. Poelma DL, Zimmermann LJ, Scholten HH et al (2002) In vivo and in vitro uptake of surfactant lipids by alveolar type II cells and macrophages. Am J Physiol Lung Cell Mol Physiol 283:L648-L654
88. Hunt AN, Kelly FJ, Postle AD (1991) Developmental variation in whole human lung phosphatidylcholine molecular species: a comparison with guinea pig and rat. Early Hum Dev 25:157-171
89. Possmayer F, Yu SH, Weber JM et al (1984) Pulmonary surfactant. Can J Biochem Cell Biol 62:1121-1133
90. Lewis JF, Jobe AH (1993) Surfactant and the adult respiratory distress syndrome. Am Rev Respir Dis 147:218-233
91. Lewis JF, Veldhuizen RA (1995) Factors influencing efficacy of exogenous surfactant in acute lung injury. Biol Neonate 67 Suppl 1:48-60
92. Bates SR, Ibach PB, Fisher AB (1989) Phospholipids co-isolated with rat surfactant protein C account for the apparent protein-enhanced uptake of liposomes into lung granular pneumocytes. Exp Lung Res 15:695-708
93. Chander A, Claypool WD, Jr., Strauss JF 3rd et al (1983) Uptake of liposomal phosphatidylcholine by granular pneumocytes in primary culture. Am J Physiol 245:C397-C404
94. Quintero OA, Wright JR (2000) Metabolism of phosphatidylglycerol by alveolar macrophages in vitro. Am J Physiol Lung Cell Mol Physiol 279:L399-L407
95. Greene KE, Wright JR, Steinberg KP et al (1999) Serial changes in surfactant-associated proteins in lung and serum before and after onset of ARDS. Am J Respir Crit Care Med 160:1843-1850
96. Gregory TJ, Longmore WJ, Moxley MA et al (1991) Surfactant chemical composition and biophysical activity in acute respiratory distress syndrome. J Clin Invest 88:1976-1981
97. Rice WR, Sarin VK, Fox JL et al (1989) Surfactant peptides stimulate uptake of phosphatidylcholine by isolated cells. Biochim Biophys Acta 1006:237-245
98. Baritussio A, Alberti A, Quaglino D et al (1994) SP-A, SP-B, and SP-C in surfactant subtypes around birth: reexamination of alveolar life cycle of surfactant. Am J Physiol 266:L436-L447
99. Baritussio A, Benevento M, Pettenazzo A et al (1989) The life cycle of a low-molecular-weight protein of surfactant (SP-C) in 3-day-old rabbits. Biochim Biophys Acta 1006:19-25
100. Rice WR, Sarin VK, Fox JL et al (1989) Surfactant peptides stimulate uptake of phosphatidylcholine by isolated cells. Biochim Biophys Acta 1006:237-245
101. Lewis JF, Brackenbury A (2003) Role of exogenous surfactant in acute lung injury. Crit Care Med 31:S324-S328
102. Miles PR, Wright JR, Bowman L et al (1983) Incorporation of [3H]palmitate into disaturated phosphatidylcholines in alveolar type II cells isolated by centrifugal elutriation. Biochim Biophys Acta 753:107-118
103. Bourbon JR, Chailley-Heu B, Gautier B (1997) The exogenous surfactant Curosurf enhances phosphatidylcholine content in isolated type II cells. Eur Respir J 10:914-919

104. Oetomo SB, Lewis J, Ikegami M et al (1990) Surfactant treatments alter endogenous surfactant metabolism in rabbit lungs. J Appl Physiol 68:1590-1596
105. Ikegami M, Jobe A, Yamada T et al (1989) Surfactant metabolism in surfactant-treated preterm ventilated lambs. J Appl Physiol 67:429-437
106. Bunt JE, Carnielli VP, Janssen DJ et al (2000) Treatment with exogenous surfactant stimulates endogenous surfactant synthesis in premature infants with respiratory distress syndrome. Crit Care Med 28:3383-3388
107. Pettenazzo A, Ikegami M, Seidner S et al (1988) Clearance of surfactant phosphatidylcholine from adult rabbit lungs. J Appl Physiol 64:120-127
108. Pettenazzo A, Jobe A, Humme J et al (1988) Clearance of surfactant phosphatidylcholine via the upper airways in rabbits. J Appl Physiol 65:2151-2155
109. Pettenazzo A, Jobe A, Ikegami M et al (1989) Clearance of phosphatidylcholine and cholesterol from liposomes, liposomes loaded with metaproterenol, and rabbit surfactant from adult rabbit lungs. Am Rev Respir Dis 139:752-758
110. Pettenazzo A, Jobe A, Ikegami M et al (1989) In vivo clearance of natural and modified surfactant. Eur Respir J Suppl 3:S13-S15
111. Walther FJ, Hernandez-Juviel J, Bruni R et al (1998) Protein composition of synthetic surfactant affects gas exchange in surfactant-deficient rats. Pediatr Res 43:666-673
112. Veldhuizen EJ, Waring AJ, Walther FJ et al (2000) Dimeric N-terminal segment of human surfactant protein B (dSP-B(1-25)) has enhanced surface properties compared to monomeric SP-B(1-25). Biophys J 79:377-384
113. Walther FJ, Hernandez-Juviel JM, Gordon LM et al (2002) Dimeric surfactant protein B peptide sp-b(1-25) in neonatal and acute respiratory distress syndrome. Exp Lung Res 28:623-640
114. Robertson B, Johansson J, Curstedt T (2000) Synthetic surfactants to treat neonatal lung disease. Mol Med Today 6:119-124
115. Walther FJ, Gordon LM, Zasadzinski JA et al (2000) Surfactant protein B and C analogues. Mol Genet Metab 71:342-351
116. Walther FJ, Hernandez-Juviel JM, Mercado PE et al (2002) Surfactant with SP-B and SP-C analogues improves lung function in surfactant-deficient rats. Biol Neonate 82:181-187
117. Hafner D, Germann PG, Hauschke D (1998) Effects of rSP-C surfactant on oxygenation and histology in a rat-lung-lavage model of acute lung injury. Am J Respir Crit Care Med 158:270-278
118. Hafner D, Germann PG, Hauschke D (1998) Comparison of rSP-C surfactant with natural and synthetic surfactants after late treatment in a rat model of the acute respiratory distress syndrome. Br J Pharmacol 124:1083-1090
119. King RJ, Carmichael MC, Horowitz PM (1983) Reassembly of lipid-protein complexes of pulmonary surfactant. Proposed mechanism of interaction. J Biol Chem 258:10672-10680
120. King RJ (1984) Lipid-apolipoprotein interactions in surfactant studied by reassembly. Exp Lung Res 6:237-253
121. Yu SH, Possmayer F (1988) Comparative studies on the biophysical activities of the low-molecular-weight hydrophobic proteins purified from bovine pulmonary surfactant. Biochim Biophys Acta 961:337-350
122. Bachurski CJ, Pryhuber GS, Glasser SW et al (1995) Tumor necrosis factor-alpha inhibits surfactant protein C gene transcription. J Biol Chem 270:19402-19407
123. Ballard PL, Liley HG, Gonzales LW et al (1990) Interferon-gamma and synthesis of surfactant components by cultured human fetal lung. Am J Respir Cell Mol Biol 2:137-143

124. Dhar V, Hallman M, Lappalainen U et al (1997) Interleukin-1 alpha upregulates the expression of surfactant protein-A in rabbit lung explants. Biol Neonate 71:46-52
125. Whitsett JA, Clark JC, Wispe JR et al (1992) Effects of TNF-alpha and phorbol ester on human surfactant protein and MnSOD gene transcription in vitro. Am J Physiol 262:L688-L693
126. Glumoff V, Vayrynen O, Kangas T et al (2000) Degree of lung maturity determines the direction of the interleukin-1- induced effect on the expression of surfactant proteins. Am J Respir Cell Mol Biol 22:280-288