A novel procedure for the rapid isolation of surfactant protein A with retention of its alveolar-macrophage-stimulating properties

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Previous studies have shown that surfactant protein A (SP-A) derived from alveolar-proteinosis patients activates rat alveolar macrophages. However, it is not known if normal rat, dog and human SP-A can also stimulate alveolar macrophages. As alveolar-proteinosis SP-A has a slightly different structure from ordinary SP-A, it would be possible that the ascribed alveolar-macrophage-stimulating properties of SP-A are restricted to alveolar-proteinosis SP-A. To clarify this issue, we isolated SP-A from normal rat and dog pulmonary surfactants, using the same isolation technique commonly used for the isolation of alveolar-proteinosis SP-A, i.e. by butanol precipitation. In contrast with human alveolar-proteinosis SP-A, rat and dog SP-A obtained thus could not activate rat alveolar macrophages to produce oxygen radicals or enhance the phagocytosis of fluorescein isothiocyanate-labelled herpes simplex virus. However, rat, dog

and normal human SP-A isolated by a novel method, involving extraction from pulmonary surfactant by using *n*-octyl β -D-glucopyranoside and subsequent purification by cation-exchange chromatography, were able to elicit an oxidative burst in rat as well as normal human alveolar macrophages. In addition, dog and rat SP-A obtained thus stimulated the phagocytosis of herpes simplex virus by rat alveolar macrophages. These findings indicate that normal human, rat and dog SP-A have the same alveolar-macrophage-stimulating properties as human alveolar proteinosis SP-A. Dog and rat SP-A isolated by this novel method had the same Ca^{2+} -dependent self-aggregation and lipid-aggregation properties as SP-A isolated by butanol precipitation. The new and milder isolation procedure yielded SP-A of high purity, as judged by SDS/PAGE and ELISA.

INTRODUCTION

Pulmonary surfactant protein A (SP-A), one of the most abundant proteins of pulmonary surfactant, is synthesized and secreted by alveolar type II cells [1–4]. Under denaturing and reducing conditions the molecular mass of SP-A is 28000–35000 Da, depending on the degree of glycosylation [3,5]. The C-terminus has sequence similarity with a number of Ca^{2+} -dependent lectins such as surfactant protein D and the plasma proteins mannose-binding protein and conglutinin [1,6,7]. The native form of SP-A is assembled into a complex of approx. 18 similar subunits [8–10], resembling the structure of C1q of the complement system [8].

Its structural similarities to C1q, conglutinin and mannose-binding proteins, which were shown to be involved in the host-defence system in the circulation, suggested a similar role for SP-A in the alveoli. Indeed, SP-A derived from alveolar-proteinosis patients (AP-SP-A) was shown to stimulate rat alveolar macrophages to produce oxygen radicals [11], and to enhance the phagocytosis of serum-opsonized bacteria [11] and non-opsonized viruses [12,13]. However, there are small structural differences between AP-SP-A and SP-A obtained from healthy humans [14], rats and dogs [9,10]. This implies that findings with AP-SP-A cannot be directly extrapolated to normal SP-A. In an attempt to clarify this issue, we isolated SP-A from pulmonary

surfactant derived from rats and dogs, using the same isolation procedure described for AP-SP-A, i.e. butanol precipitation. As will be demonstrated in this study, butanol precipitation of rat and dog SP-A destroys the rat-alveolar-macrophage-stimulating properties of SP-A, measured as the production of oxygen radicals and phagocytosis of herpes simplex virus by these cells. We report a new and milder isolation procedure for rat, dog and normal human SP-A, yielding SP-A that is able to activate rat as well as human alveolar macrophages.

MATERIALS AND METHODS

Isolation of rat and dog pulmonary surfactants

Pulmonary surfactant was isolated from the broncho-alveolar lavages from dogs and rats as described previously [5,11–13]. Surfactant was suspended in distilled water, with a Dounce homogenizer.

Isolation of SP-A by *n*-octyl β -D-glucopyranoside (OGP) extraction

Dog pulmonary surfactant (2.2 ml; 4.7 mg of protein and 9.8 mg of phospholipids per ml of distilled water) was suspended in 200 ml of extraction buffer, consisting of 5 mM Tris and 6 mM OGP, pH 7.8, with a Dounce homogenizer. The mixture was

Abbreviations used: SP-A, surfactant protein A; OGP, *n*-octyl β -D-glucopyranoside; AP-SP-A, SP-A derived from alveolar-proteinosis patients; OGP-SP-A, SP-A isolated by OGP extraction; BuOH-SP-A, SP-A isolated by butanol precipitation; OGP-BuOH SP-A, SP-A isolated by OGP extraction and subjected to butanol precipitation.

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incubated for 2 h at room temperature with magnetic stirring. Subsequently, the mixture was centrifuged for 1 h at 100 000 *g*. The supernatant was removed, and 20 ml of 200 mM ethanolamine buffer, pH 9.2 (final concn. 20 mM) was added to this supernatant. The extraction mixture was filtered through a 0.45 μ m-pore filter and applied to a Mono Q column (Pharmacia, Uppsala, Sweden) of an HPLC system (Pharmacia) by using a Superloop (50 ml; Pharmacia) in two successive steps (applied volume 110 ml per step) at a flow rate of 1 ml/min at room temperature. The column was washed with 4 bed volumes of 20 mM ethanolamine buffer, pH 9.2, containing 6 mM OGP (buffer A). The bound proteins were eluted with a linear gradient of a buffer consisting of 20 mM ethanolamine, 6 mM OGP and 1 M NaCl, pH 9.2 (buffer B), as depicted in Figure 1. Subsequently the remaining extract was applied to the column, and the bound proteins were eluted in the manner described. The SP-A fractions were collected, pooled and dialysed against four changes of 10 mM boric acid, pH 9.0, for 48 h at 4 °C. SP-A was stored in small portions at -70 °C.

For the preparation of OGP-BuOH SP-A (SP-A isolated by OGP extraction and subjected to butanol precipitation), 0.5 mg of dog SP-A purified by the OGP extraction was added to 3 ml of butanol and incubated for 30 min. Subsequently, the precipitated SP-A was collected by centrifugation (10 000 *g*; 30 min). The supernatant was discarded, and the pellet was dried under N₂ gas. The dried pellet was suspended in 10 mM boric acid, pH 9.0, and dialysed against the same buffer. The undissolved material was removed by centrifugation (100 000 *g*; 30 min). The overall yield of SP-A by the butanol precipitation was 53 % as determined by the ELISA specific for SP-A. SP-A was stored at -70 °C.

Isolation of human AP-SP-A

SP-A was isolated from the alveolar lavage of alveolar-proteinosis patients as described elsewhere [11–13]. SP-A at 2.2 mg/ml of 5 mM Tris buffer, pH 7.4, was stored in small batches at -70 °C until further use.

Isolation of rat and dog SP-A by butanol precipitation

This was performed as described elsewhere [5].

Isolation of rat alveolar macrophages

Rat alveolar macrophages were isolated from male specific-pathogen-free Wistar rats (200–225 g) as described previously [11]. The macrophages were suspended in 140 mM NaCl/5 mM KCl/2.5 mM Na₂HPO₄/10 mM Hepes/2 mM MgSO₄/6 mM glucose/2 mM CaCl₂ buffer, pH 7.4, supplemented with 0.1 % (w/v) gelatin, at a concentration of 2×10^6 cells/ml.

Broncho-alveolar lavage of healthy human volunteers

Broncho-alveolar lavage of healthy volunteers (male and female; ages 22–39) was performed after premedication with atropine and local lidocaine anaesthesia. A flexible bronchoscope was wedged into the middle lobe of the right lung. Five successive portions of 40 ml of 0.15 M NaCl, warmed to 37 °C, were instilled. Approx. 50 % of the instilled fluid was recovered, and collected in silicone-treated glassware. Alveolar macrophages were collected as described under 'Isolation of rat alveolar macrophages'. The pulmonary surfactant of these volunteers was isolated by the same method as for rat and dog surfactant. SP-A was isolated from surfactant by the OGP-extraction method as described above.

Chemiluminescence assay

The generation of oxygen radicals by rat alveolar macrophages was monitored with the lucigenin-dependent chemiluminescence assay as described in [11]. Briefly, the chemiluminescence response of 3.5×10^5 alveolar macrophages was determined in the presence of lucigenin (250 μ M) after stimulation with human AP-SP-A (10 μ g/ml), normal human SP-A (10 μ g/ml), dog or rat SP-A (5 μ g/ml), or dog or rat surfactant. The assay volume was 0.4 ml. Chemiluminescence was measured at 37 °C in a luminometer for 3 s every 2 min over a 45 min period. Peak signals of the chemiluminescence responses were used to compare the various experimental conditions, and were expressed as a percentage of the peak responses of the macrophages in the absence of stimuli.

Fluorimetric phagocytosis assay

The phagocytosis of fluorescein isothiocyanate (FITC)-labelled herpes simplex virus type 1 (HSV-1) by rat alveolar macrophages was recorded by using the fluorimetric phagocytosis assay as described previously [13]. Briefly, alveolar macrophages (0.25 ml; 2×10^6 /ml) were incubated with 0.03 ml of purified FITC-labelled herpes simplex virus and various concentrations of SP-A (final volume 0.4 ml) at 37 °C for 30 min. Cell-associated fluorescence, a measure for the phagocytosis of herpes virus, was determined with a FACscan flow cytometer (Becton Dickinson, Mountain View, CA, U.S.A.). Phagocytosis of herpes virus by alveolar macrophages in the presence of SP-A was expressed as a percentage of the phagocytosis of the virus in the absence of stimuli.

Determination of dog SP-A

The content of SP-A in the various steps of the purification of dog SP-A was determined by sandwich ELISA using the ascites fluid of a murine monoclonal antiserum directed against dog SP-A and the IgG fraction of a rabbit polyclonal antiserum directed against dog SP-A. A 0.1 ml portion of rabbit anti-(dog SP-A) IgG fraction (10 μ g/ml of 0.1 M NaHCO₃, pH 9.6) was incubated overnight at 4 °C in wells of microtitre plates (Nunc-Immuno Plate MaxiSorp; Nunc A/S, Roskilde, Denmark). The wells were incubated with PBS (0.2 ml/well) containing 0.1 M EDTA and 2 % (w/v) Protifar (albumin substitute, from Nutricia, Zoetermeer, The Netherlands), pH 7.2, for 1 h at room temperature. The wells were washed three times with 0.05 % (w/v) Tween-20 in PBS. Then 0.1 ml of purified dog SP-A standard (purified by butanol precipitation) or various dilutions of samples in 0.01 M EDTA and 2 % Protifar buffered with 0.005 M Tris/HCl, pH 7.4, were added to each well and incubated for 1 h at room temperature. The wells were washed, and 0.1 ml of murine ascites-fluid anti-(dog SP-A) antiserum (diluted 1:10 000 in PBS containing 0.01 M EDTA and 2 % Protifar, pH 7.2) was added to each well and incubated for 1 h at room temperature. After three washes, 0.1 ml of rabbit anti-mouse IgG conjugated with horseradish peroxidase (diluted 1:20 000 in EDTA/Protifar/PBS; Nordic Immunology, Tilburg, The Netherlands) was added to each well and allowed to incubate for 1 h at room temperature. The wells were washed, and 0.1 ml of substrate solution, consisting of 0.02 % (w/v) 3,5,3',5'-tetramethylbenzidine, 0.003 % H₂O₂ and 0.1 M phosphate/citrate buffer, pH 4.0, was added to each well and incubated for 20 min at room temperature. The reaction was terminated by addition of 0.05 ml of 2 M H₂SO₄ per well. The A₄₅₀ was determined with an EASY reader (EAR 400; SLT-Lab instruments, Vienna, Austria). The assay is capable of accurately measuring SP-A in concentrations of 2–75 ng/ml. However, for the determination of SP-A in

surfactant, 0.006 M deoxycholate was added to the surfactant before dilution of the surfactant for the assessment of SP-A by ELISA. The presence of deoxycholate was necessary to dissociate SP-A from the lipids. In that way, accurate measurement of the SP-A content in surfactant was achieved. Control experiments performed with purified dog SP-A added to surfactant lipids indicated that approx. 95 % of the added SP-A could be detected in the ELISA in the presence of 0.006 M deoxycholate. For the determination of SP-A in the OGP extract or in the other steps of the purification procedure, the presence of deoxycholate was not necessary (results not shown), and it was therefore omitted.

Aggregation assays

Ca²⁺-dependent self-association of SP-A was investigated by turbidity measurements at 400 nm on an Aminco DW-2a spectrophotometer as described previously [12]. Briefly, sample and reference cuvettes contained SP-A (10 µg), 0.1 M NaCl and 0.005 M Tris, pH 7.4. Aggregation was initiated by addition of CaCl₂ (final concn. 0.005 M) to the sample cuvette. To study the lipid aggregation, vesicles (dipalmitoyl phosphatidylcholine/phosphatidylglycerol, 7:3, w/w) were added to the cuvettes as described elsewhere [15].

Electrophoresis

Protein electrophoresis was performed by SDS/PAGE as described by Laemmli [16], in 12 % gels.

Protein

Protein concentrations were determined by the method of Lowry et al. [17], with BSA as standard.

RESULTS AND DISCUSSION

Isolation of SP-A by OGP extraction

Approx. 53 % of SP-A can be extracted from surfactant in the presence of 6 mM OGP (Table 1). Neither higher OGP concentrations (up to 24 mM) nor longer incubation times further increased the yield of SP-A extracted from surfactant (results not shown). Also, a second extraction of the surfactant with OGP buffer yielded only an additional 8 % of SP-A, and was therefore omitted from the isolation procedure (results not shown). For an optimal extraction of SP-A from surfactant, the best results were obtained when surfactant was suspended in extraction buffer at a protein concentration of no more than 50 µg/ml.

Loading of the surfactant OGP extract on the Mono Q column and elution of the bound proteins was performed at pH 9.2 and in the presence of OGP in order to prevent

Figure 1 Chromatography of OGP extract of dog surfactant on a Mono Q column

(a) Profile of the elution of proteins using buffer B (see the Materials and methods section). Dashed line reflects the percentage of buffer B, and the continuous line the A_{280} . (b) Samples of the eluted proteins (fractions 1–6) were subjected to reduced SDS/PAGE. The amount of protein loaded in each track was: fractions 1–3, 2 µg; fractions 4 and 5, 8 µg; and fraction 6, 6 µg.

Figure 2 Comparison of the molecular masses of dog SP-A isolated by butanol precipitation and OGP extraction

SP-A isolated by OGP extraction (lane a, reduced; lane c, unreduced) and SP-A isolated by butanol precipitation (lane b, reduced; lane d, unreduced).

Table 1 Purification of SP-A from dog surfactant

Results are shown of a representative purification procedure of dog SP-A out of four procedures. 'Ag in mg' is the amount of SP-A in mg as determined by ELISA.

Fraction	Total protein (mg)	Total SP-A (Ag in mg)	Yield of SP-A (%)
Surfactant (2.2 ml)	10.34	2.86	100
OGP-extract (200 ml)	6.51	1.53	53.5
After HPLC and dialysis	1.10	1.26	44.1

aggregation of SP-A, which occurs readily at high SP-A concentrations and lower pH [15]. SP-A was eluted from the column at a buffer-B concentration of approx. 45 %, as a single peak (Figures 1a and 1b; fraction 6). The SP-A thus obtained was pure as judged by SDS/PAGE (Figure 1b) and ELISA (Table 1), and consists of a triplet with apparent molecular masses around 35000 Da, as determined by reduced SDS/PAGE (Figure 2, lane a). In the absence of reducing substances, SP-A had an apparent molecular mass of 60000 Da (Figure 2, lane c). These molecular masses for the SP-A isolated by OGP extraction (OGP-SP-A) are the same as those for SP-A isolated by the commonly used

Figure 3 Comparison of the self-aggregation and lipid aggregation of SP-A isolated either by butanol precipitation or by OGP extraction

Dog SP-A (10 μ g/ml) isolated either by butanol precipitation (dashed line) or by OGP extraction (continuous line) were incubated (final volume 1 ml) in 0.1 M NaCl/5 mM Tris, pH 7.4. Self-aggregation was initiated by addition of 5 mM CaCl_2 (arrow 1). Lipid aggregation was initiated by addition of 100 μ g of phospholipid vesicles (arrow 2).

butanol-precipitation technique (BuOH-SP-A) (Figure 2, lanes b and d) and in agreement with the observed molecular masses of SP-A isolated by the latter technique by others [3,9,10,14]. Also, the apparent molecular mass of SP-A isolated by these two different methods under non-denaturing circumstances was the same, approx. 650000, as determined by gel filtration on a Superose 6 column (results not shown).

Influence of the isolation procedure on the properties of SP-A

In the presence of Ca^{2+} ions SP-A was reported to aggregate [18]. Self-association is a prerequisite for lipid aggregation and depends on a structurally intact collagen domain [15,18]. Both OGP-SP-A and BuOH-SP-A from dog surfactant were able to self-associate in the presence of Ca^{2+} ions (Figure 3). In addition, OGP- and BuOH-SP-A demonstrated similar lipid-aggregation curves. SP-A-mediated lipid aggregation requires lipid and Ca^{2+} binding and interaction of the carbohydrate-binding domains of SP-A with the carbohydrate moieties of SP-A [18]. Hence, the butanol-precipitation procedure and the OGP-extraction method for SP-A isolation do not seem to affect the domains of SP-A involved in the processes described above. Similar results were obtained when these experiments were repeated with OGP-SP-A and BuOH-SP-A isolated from rat surfactant (not shown).

Dog as well as rat surfactant enhanced the production of oxygen radicals by rat alveolar macrophages (Table 2). Previously, we were able to demonstrate, using monospecific antibodies against SP-A, that the component in surfactant responsible for the activation of alveolar macrophages is SP-A [11]. However, in contrast with SP-A isolated by OGP extraction, dog and rat SP-A isolated by butanol precipitation from the same surfactant neither induced a chemiluminescence response in rat alveolar macrophages nor stimulated the phagocytosis of herpes simplex virus by these cells (Table 2). These results suggest that isolating SP-A from surfactant by the butanol-precipitation technique destroys the macrophage-stimulating properties of SP-A. Though SP-A acts as an opsonin in the phagocytosis of herpes simplex virus [12,13], and therefore requires interaction of SP-A with the virus as well as with the macrophages, theoretically butanol extraction may also lead to inactivation of the herpes simplex binding site on SP-A.

It may be argued that, by using the different isolation methods, butanol precipitation and OGP extraction, two different forms of

Table 2 Influence of rat and dog total surfactant and SP-A on rat alveolar macrophage functions

Rat alveolar macrophages were incubated either with SP-A isolated by OGP extraction (OGP-SP-A) or isolated by butanol precipitation (BuOH), or SP-A isolated by OGP extraction and subsequently subjected to butanol precipitation (OGP + BuOH), or with whole surfactant. The results are expressed as percentages of the response in the absence of stimuli (SP-A or surfactant). The observations are means $\pm$ S.E.M. of the number of experiments indicated (*n*). Statistical significance: $P < 0.05$ (Student's *t* test). NS, not significant. ND, not determined.

	Oxygen-radical production	Herpes simplex virus phagocytosis
Dog surfactant	310 $\pm$ 18 (<i>n</i> = 3; $P < 0.05$)	ND
Dog SP-A (OGP)	371 $\pm$ 14 (<i>n</i> = 4; $P < 0.05$)	443 $\pm$ 61 (<i>n</i> = 3; $P < 0.05$)
Dog SP-A (BuOH)	101 $\pm$ 4 (<i>n</i> = 5; NS)	103 $\pm$ 22 (<i>n</i> = 3; $P < 0.05$)
Dog SP-A (OGP + BuOH)	134 $\pm$ 24 (<i>n</i> = 3; NS)	ND
Rat surfactant	287 $\pm$ 17 (<i>n</i> = 4; $P < 0.05$)	ND
Rat SP-A (OGP)	263 $\pm$ 12 (<i>n</i> = 4; $P < 0.05$)	390 $\pm$ 20 (<i>n</i> = 4; $P < 0.05$)
Rat SP-A (BuOH)	97 $\pm$ 5 (<i>n</i> = 5; NS)	110 $\pm$ 15 (<i>n</i> = 3; NS)

Table 3 Influence of dog, rat and human SP-A on the oxygen-radical production by rat and human alveolar macrophages

The results are expressed as percentages of the response in the absence of stimuli (SP-A). The observations are means $\pm$ S.E.M. of the number of experiments indicated (*n*). Statistical significance: $P < 0.05$ (Student's *t* test). ND, not determined.

	Alveolar macrophages	
	Rat	Human
Dog SP-A	371 $\pm$ 14 (<i>n</i> = 4; $P < 0.05$)	217 $\pm$ 25 (<i>n</i> = 8; $P < 0.001$)
Rat SP-A	263 $\pm$ 12 (<i>n</i> = 4; $P < 0.05$)	ND
Human SP-A	205 $\pm$ 13 (<i>n</i> = 3; $P < 0.05$)	226 $\pm$ 40 (<i>n</i> = 3; $P < 0.05$)
Human AP-SP-A	293 $\pm$ 45 (<i>n</i> = 4; $P < 0.05$)	274 $\pm$ 36 (<i>n</i> = 11; $P < 0.001$)

SP-A are isolated from surfactant: one form isolated by butanol precipitation, which has the characteristics of SP-A such as the molecular mass, the self-aggregation and the lipid-binding properties, but is not able to activate alveolar macrophages, and another form isolated by OGP extraction, which has all the above-mentioned SP-A properties, including stimulation of alveolar macrophages. This seems unlikely, since SP-A isolated by OGP extraction loses its property to activate rat alveolar macrophages after subsection to the butanol-precipitation technique (Table 2).

Stimulation of the oxidative burst in alveolar macrophages by SP-A

SP-A isolated from surfactant derived from healthy volunteers, rats and dogs by the OGP extraction technique induced an oxidative burst in rat alveolar macrophages (Table 3). In addition, dog and human SP-A (not enough human macrophages were available to test rat SP-A) also elicited a chemiluminescence response in human alveolar macrophages (Table 3). However, human as well as dog SP-A isolated by butanol precipitation did

not stimulate the oxygen radical production by human alveolar macrophages (results not shown). These results may suggest that the stimulation of alveolar macrophages by SP-A is not dependent on the species from which SP-A or alveolar macrophages are derived.

We previously demonstrated that SP-A derived from alveolar-proteinosis patients (AP-SP-A) stimulated rat alveolar macrophages to enhanced phagocytosis of serum-opsonized bacteria [11], the production of oxygen radicals [11] and phagocytosis of herpes simplex virus [12,13]. However, Weber et al. [19] found no stimulation of the lucigenin-dependent chemiluminescence response when dog broncho-alveolar phagocytes were incubated with dog SP-A. In addition, Katsura et al. [20] also did not detect an enhancement of the oxygen-radical production of rat alveolar macrophages by rat SP-A. These authors [19,20] suggested that the stimulation of alveolar macrophages by SP-A reported by van Iwaarden et al. [11] is caused by either the use of heterologous test system, i.e. human SP-A and rat alveolar macrophages, or by special properties of AP-SP-A which ordinary SP-A does not possess. Our present study offers an alternative explanation. The SP-A used by Weber et al. [19] and Katsura et al. [20] was isolated by butanol precipitation, and had probably lost its macrophage-stimulating properties as a result of that procedure.

AP-SP-A differs from SP-A from normal humans [14], rats and dogs [9,10]. It contains a dimer with an apparent molecular mass of approx. 70000 Da, which is not present in normal human, rat or dog SP-A, as can be demonstrated by reduced SDS/PAGE. Under non-denaturing and low-ionic-strength conditions AP-SP-A tends to be more aggregated than dog SP-A [10].

Self-association of SP-A in the presence of Ca^{2+} or SP-A bound to lipids yields SP-A which is less sensitive to ozone damage [21]. Perhaps by analogy with these ozone experiments, AP-SP-A, which is more aggregated than normal SP-A, is less susceptible to butanol, and is therefore able to stimulate alveolar macrophages, whereas dog or rat SP-A loses its alveolar-macrophage-stimulating properties after butanol treatment. However, further studies are warranted to clarify exactly the differences between AP-SP-A and normal SP-A with regard to the effect of butanol on its alveolar-macrophage stimulation properties.

In conclusion, this study demonstrates that SP-A can be isolated from normal rat, dog and human surfactant by using the

OGP-extraction method to study the interactions of SP-A with alveolar macrophages. For the isolation of functionally active AP-SP-A, the butanol-precipitation technique seems more appropriate, since larger quantities of SP-A can be isolated at a time than by the OGP-extraction method.

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REFERENCES

- 1 Phelps, D. S. and Floros, J. (1988) *Am. Rev. Respir. Dis.* **137**, 939–942
- 2 Voorhout, W. F., Veenendaal, T., Kuroki, Y., Ogasawara, Y., van Golde, L. M. G. and Geuze, H. J. (1992) *J. Histochem. Cytochem.* **40**, 1589–1597
- 3 Possmayer, F. (1988) *Am. Rev. Respir. Dis.* **138**, 990–998
- 4 Hawgood, S. (1989) *Am. J. Physiol.* **257**, L13–L22
- 5 Benson, B., Hawgood, S. and Schilling, J. (1985) *Proc. Natl. Acad. Sci. U.S.A.* **82**, 6379–6383
- 6 Drickamer, K. (1988) *J. Biol. Chem.* **263**, 9557–9560
- 7 Thiel, S. and Reid, K. B. M. (1989) *FEBS Lett.* **250**, 78–84
- 8 Voss, T., Eistetter, H., Schäfer, K. P. and Engel, J. (1988) *J. Mol. Biol.* **201**, 219–227
- 9 King, R. J., Simon, D. and Horowitz, P. M. (1989) *Biochim. Biophys. Acta* **1001**, 294–301
- 10 Haagsman, H. P., White, R. T., Schilling, J., Lau, K., Benson, B. J., Hawgood, S. and Clements, J. A. (1989) *Am. J. Physiol.* **257**, L421–L429
- 11 Van Iwaarden, F., Welmers, B., Verhoef, J., Haagsman, H. P. and Van Golde, L. M. G. (1990) *Am. J. Respir. Cell Mol. Biol.* **2**, 91–98
- 12 Van Iwaarden, J. F., Van Strijp, J. A. G., Visser, H., Haagsman, H. P., Verhoef, J. and Van Golde, L. M. G. (1992) *J. Biol. Chem.* **267**, 25039–25043
- 13 Van Iwaarden, J. F., Van Strijp, J. A. G., Ebskamp, M. J. M., Welmers, A. C., Verhoef, J. and Van Golde, L. M. G. (1991) *Am. J. Physiol.* **261**, L204–L209
- 14 Voss, T., Schäfer, K. P., Nielson, P. F., Schäfer, A., Maier, C., Hannappel, E., Maasen, J., Landis, B., Klemm, K. and Przybylski, M. (1992) *Biochim. Biophys. Acta* **1138**, 261–267
- 15 Haagsman, H. P., Sargeant, T., Hauschka, P. V., Benson, B. J. and Hawgood, S. (1990) *Biochemistry* **29**, 8894–8900
- 16 Laemmli, U. K. (1970) *Nature (London)* **227**, 680–685
- 17 Lowry, O. H., Rosebrough, N. J., Farr, A. L. and Randall, R. J. (1951) *J. Biol. Chem.* **193**, 265–275
- 18 Haagsman, H. P., Elfring, R. H., van Buel, B. L. M. and Voorhout, W. F. (1991) *Biochem. J.* **275**, 273–276
- 19 Weber, H., Heilmann, F., Meyer, B. and Maier, K. L. (1990) *FEBS Lett.* **270**, 90–94
- 20 Katsura, H., Kawada, H. and Konno, K. (1993) *Am. J. Respir. Cell Mol. Biol.* **9**, 520–525
- 21 Oosting, R. S., van Greevenbroek, M. M. J., Verhoef, J., van Golde, L. M. G. and Haagsman, H. P. (1991) *Am. J. Physiol.* **261**, L77–L83