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Rapid Communication

Identification of a Cell Membrane Protein That Binds Alveolar Surfactant

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Alveolar surfactants are complex mixtures of proteins and phospholipids produced by type II alveolar cells and responsible for lowering pulmonary surface tension. The processes by which surfactant is produced and exported and by which its production by pulmonary cells is regulated are not well understood. This study was designed to identify a cellular receptor for surfactant constituents. To do so, monoclonal anti-idiotypic antibodies directed against antibodies to porcine and rabbit surfactant proteins were prepared. These monoclonal anti-idiotypic antibodies bind both alveolar lining and bronchial epithelial cells in rabbit, porcine, and human lungs. Macrophages and other nonepithelial cells do not react with these antibodies. Western blot analysis indicates that both A2R and A2C recognize the same proteins in both pig and rabbit lungs: a 30-kd protein and additional proteins at 52 and 60 kd. Preincubating lung wash cells with A2C or A2R prevents binding of porcine or rabbit surfactant preparations, respectively, by these cells. Preincubating frozen sections of lung tissue with surfactant inhibits binding of A2R and A2C to the lung. Antibody directed to a cell membrane protein that recognizes alveolar surfactant may be useful in elucidating the structure and function of this receptor and in understanding the cellular physiology and pathophysiology of the surfactant system. (Am J Pathol 1991, 138:1085–1095)

Pulmonary surfactant (SRF) proteins are of three characterized varieties.[1] Surfactant protein A (SP-A) is an apoprotein of approximately 30 to 35 kd molecular weight.[2] In its secreted form, SP-A is glycosylated. It appears to stabilize tertiary and quaternary surfactant structures *in vivo* and *in vitro*, and may help protect SRF activity from inhibition by other proteins.[3] Its role in the surface activity of SRF is not yet clear. Surfactant protein B (SP-B) is 8 to 10 kd. It occurs in the alveolar fluid principally as a homodimer. In some animal species, SP-B appears to be the principal active protein in pulmonary SRF preparations.[4] Surfactant protein C (SP-C) is a very hydrophobic lipoprotein of 3 to 5 kd.[5] The function of SP-C is not entirely clear. In some preparations, SP-C plays a major role in the ability of SRF to lower surface tension.[6]

These proteins are secreted by type II alveolar pneumocytes. Then they combine with phospholipids to form a protein–phospholipid layer at the air-liquid interphase. Thus SP-A is thought to be both secreted and recycled by type II cells.[7,8] This large SRF protein probably modulates phospholipid secretion by type II cells.[9,10] Recent work suggests that SP-A binds to type II alveolar cells with high affinity *via* a specific receptor[11,12] that remains unidentified to date. Little is known about receptors for smaller SRF proteins.

Because of these indications that SRF may regulate its own metabolism by interacting with a specific receptor, and because the nature of such a receptor remains unknown, characterization of SRF receptor structure and activity remain important unresolved issues. We decided to approach this study by trying to make antibody to this receptor, and then to use this antibody to characterize the receptor, its ability to bind SRF and cells bearing this receptor. Our studies here describe the identification of one such receptor on porcine and rabbit lung cells.

Materials and Methods

Surfactant

Curosurf was the gift of Drs. B. Robertson and T. Curstedt, from the Department of Pathology, Karolinska Insti-

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tute, Stockholm, Sweden. Its protein content is well characterized: Curosurf contains a mixture of SP-B and SP-C only.[13,14] We have confirmed this observation by experiments in which we radiolabeled Curosurf and detected only proteins of 4 kd, 9 kd, and 18 kd, corresponding to SP-C, SP-B monomer, and SP-B dimer, respectively (data not shown). Rabbit SRFs were prepared by ultracentrifugation of rabbit lung lavage specimens and was given to us by Dr. T. A. Merritt. Their protein constituents are SP-A and SP-B.[15]

Iodination of Surfactant

Curosurf or RSRP was radiolabeled (^{125}I) using chloramine T.[16] Analysis of Curosurf by this technique shows that it contains proteins of approximately 4 kd, 9 kd, and 18 kd. Similar analyses of rabbit and human surfactants show proteins of 35 kd, 18 kd, and 9 kd (data not shown).

Monoclonal Antibodies to Surfactant

We have produced 15 monoclonal antibodies to Curosurf and five such monoclonal antibodies to rabbit SRF in female F344 rats (Harlen Co., Indianpolis, IN). Production, characterization, and specifities are described in a recent publication.[17] Most of these antibodies inhibit surface activity of their respective SRFs *in vitro*[18] and react with 9 kd proteins in Curosurf and rabbit SRF (RSRF), respectively. This molecular weight is consistent with SP-B. These hybridomas were passaged in medium with fetal bovine serum and separated from serum proteins by two cycles of precipitation with ammonium sulfate at 4°C.

Anti-[anti-SRF] Anti-idiotypic Antibodies

Anti-idiotype antibodies directed to anti-Curosurf and anti-rabbit SRF monoclonal antibodies[17] were raised by immunizing rats of the same strain (F344) with monoclonal antibodies. For the current studies, only monoclonal antibodies directed against the 9-kd (SP-B) component of Curosurf or the 9-kd component of RSRF (SP-B) were used to generate the anti-idiotype antibodies. In addition, we used only anti-SRF antibodies that could inactivate SRF in the pulsating bubble surfactometer assay.[17,18] Because the proteins contained in RSRF are SP-A and SP-B, and those in Curosurf are SP-B and SP-C, limiting ourselves to monoclonal antibodies that recognize 9-kd proteins in these SRF preparations and inactivate SRF functionally greatly enhances the likelihood that these original antibodies will recognize SP-B.

These anti-SRF antibodies were emulsified at 100 μg/ml in Freund's adjuvant and inoculated biweekly. After four immunizations, rats were inoculated intraperitoneally with anti-SRF antibodies in saline biweekly. They were bled before immunization and regularly thereafter. Sera were examined for anti-idiotype activity by enzyme-linked immunoassay (ELISA). After approximately 1 year of this regimen, anti-[anti-SRF] titers were first detected ($A_{405} \geq 3 \times$ background). Rats with serum antibody were boosted and their spleen cells fused to YB2/0 cells. Resulting hybridomas were cloned, subcloned, and maintained as described.[17] One clone of cells producing anti-[anti-Curosurf] monoclonal antibody (A2C) was identified, as was a single clone of cells producing anti-[anti-RSRF] (A2R). Both cell lines have been stable and productive in culture.

Pulmonary Wash Cells

Fresh pulmonary cells (hereafter lung cells) from vigorous saline lavage of porcine and rabbit lungs were prepared by centrifugation and washing with phosphate-buffered saline (PBS). Lavage cells from these animals contain a mixture of alveolar lining cells, bronchial epithelial cells, lymphocytes, and macrophages when examined by Papanicolau stain (data not shown).

Cytofluorography

Cytofluorographic analysis of lung lavage cells was performed as described in other systems.[19] Briefly, after exposure to normal rabbit serum, cells were treated with monoclonal antibody as undiluted tissue culture supernatants, followed by fluorescein isothiocynate (FITC)-conjugated rabbit anti-rat immunoglobulin G (IgG) (Miles-Yeda Ltd., Rehovot, Israel). Cytofluorographic analysis was performed using a FACS II (Becton-Dickinson Corp, Franklin Lakes, NJ).

Immunocytochemistry

Frozen sections of human, pig, and rabbit lungs and cytospin preparations of lung wash cells were treated with normal rabbit serum, followed by A2R, A2C, or control preparations, and then horseradish peroxidase (HRP)-rabbit anti-rat IgG. Reactivity was visualized as described.[20] Control preparations in all cases consisted of culture supernatants from YB2/0 cells.

Western Blotting

Determination of specific protein reactivity of the anti-idiotypic antibodies was performed by Western

blotting.[21] Lysates of alveolar wash cells were prepared using 1% NP-40 (U.S. Biochemical Corp., Cleveland, OH) in PBS. After removing debris, we reduced and electrophoresed this lysate using sodium dodecyl sulfate (SDS)-10% polyacrylamide gel electrophoresis (PAGE) and transferred to Immobilon filters (Millipore Corp., Bedford, MA).[21] Filters were exposed to A2C, A2R, or control antibodies as culture supernatants, followed by rabbit anti-rat IgG and ^{125}I-*Staphylococcus* protein A, as described.[17,18] We visualized reactivity by autoradiography.

Surfactant Binding

Lung cells at 10^7/ml PBS-0.1% NaN_3 were incubated on ice in suspension with either anti-idiotypic or control antibodies, free surfactant (1000-fold excess), or saline. They were washed and then treated with 4 ng ^{125}I-Curosurf or RSRF preparations in PBS-0.1% NaN_3, washed thoroughly, and counted in a gamma counter.

Inhibition of Antibody Binding to Lung Sections

Frozen sections of lung were preincubated for 15 minutes at room temperature with homologous surfactant in normal saline (5 mg/ml surfactant phospholipid, 50 μg/ml protein) or normal saline. Slides were then exposed to A2R and A2C followed by HRP-rabbit anti-rat IgG, as discussed above.[20] In these experiments, frozen sections were not preincubated with H_2O_2.

Results

Monoclonal Antibodies to Anti-SRF Antibodies

Monoclonal anti-[anti-Curosurf] and anti-[anti-RSRF] were produced in rats as described in Materials and Methods. One stable clone of each anti-[anti-SRF] antibody (A2C and A2R, respectively) was generated in this way. When preincubated with anti-SRF antibodies in replicate assays, these monoclonal anti-idiotype antibodies inhibited more than 75% of the binding of SRF by antibodies directed to SRF (data not shown).

Cytofluorographic Analysis of Lung Cells Using A2C and A2R

Lung lavage cells were treated with anti-[anti-SRF], anti-SRF monoclonal antibody, or saline and analyzed by cytofluorography (Figures 1,2). Compared to controls, anti-[anti-SRF] anti-idiotype antibodies recognized heterogeneous populations of cells comprising 30% of the total cells in their respective pulmonary lavage specimens.

Immunohistochemical Analysis of Cells Bound by Anti-[anti-surfactant] Antibodies

Cytospin preparations of lung cells were treated with anti-[anti-SRF] and visualized by immunoperoxidase staining. Both antibodies recognized two populations of cells (Figure 3). A2C and A2R bound alveolar lining cells, small, round cells with central nuclei. These cells stain moderately along their cell membranes and within the cytoplasm. In addition, ciliated columnar bronchial and bronchiolar lining cells are recognized by A2C and A2R. Their cilia and the basal end of the cilia at their attachment to the cytoplasm proper react very strongly. The cytoplasm of these cells generally stains weakly or not at all. Macrophages and lymphocytes are not bound by either of the monoclonal anti-idiotypic antibodies.

Frozen sections of rabbit, human and porcine lung were, similarly, stained using both antibodies. Figure 4 shows that both bronchial epithelium and the epithelial cells lining the alveoli are recognized by both of these antibodies. Control preparations did not stain lung tissue. The antigens recognized by these two antibodies are present in the lungs of all three species and are distributed similarly in all. Interestingly, although neither A2C nor A2R was raised against anti-human SRF antibody, both recognize an antigen in human lung. Our attempts to raise monoclonal anti-[anti-(human SRF)] antibodies have not been successful.

The specificity of the binding of A2C and A2R to a SRF-binding protein in lung tissues was determined further by trying to inhibit this binding with SRF. Frozen sections of lung were preincubated with a preparation of homologous surfactant at 5 mg/ml phospholipid, 50 μg/ml protein. A2R or A2C was added and visualized as before. We found that preincubation with SRF greatly diminished the ability of A2C and A2R to bind to alveolar and bronchial lining cells (Figure 5).

Western Blot Analysis

Lysates of lung cells were prepared by freezing and thawing the cells once and solubilizing them in PBS-1% NP-40. They were electrophoresed under reducing conditions in SDS-10% PAGE and transferred to Immobilon filters. Filters were treated with one or the other monoclonal antibody. Both antibodies recognize similar proteins in their respective lung preparations and in cell lysates

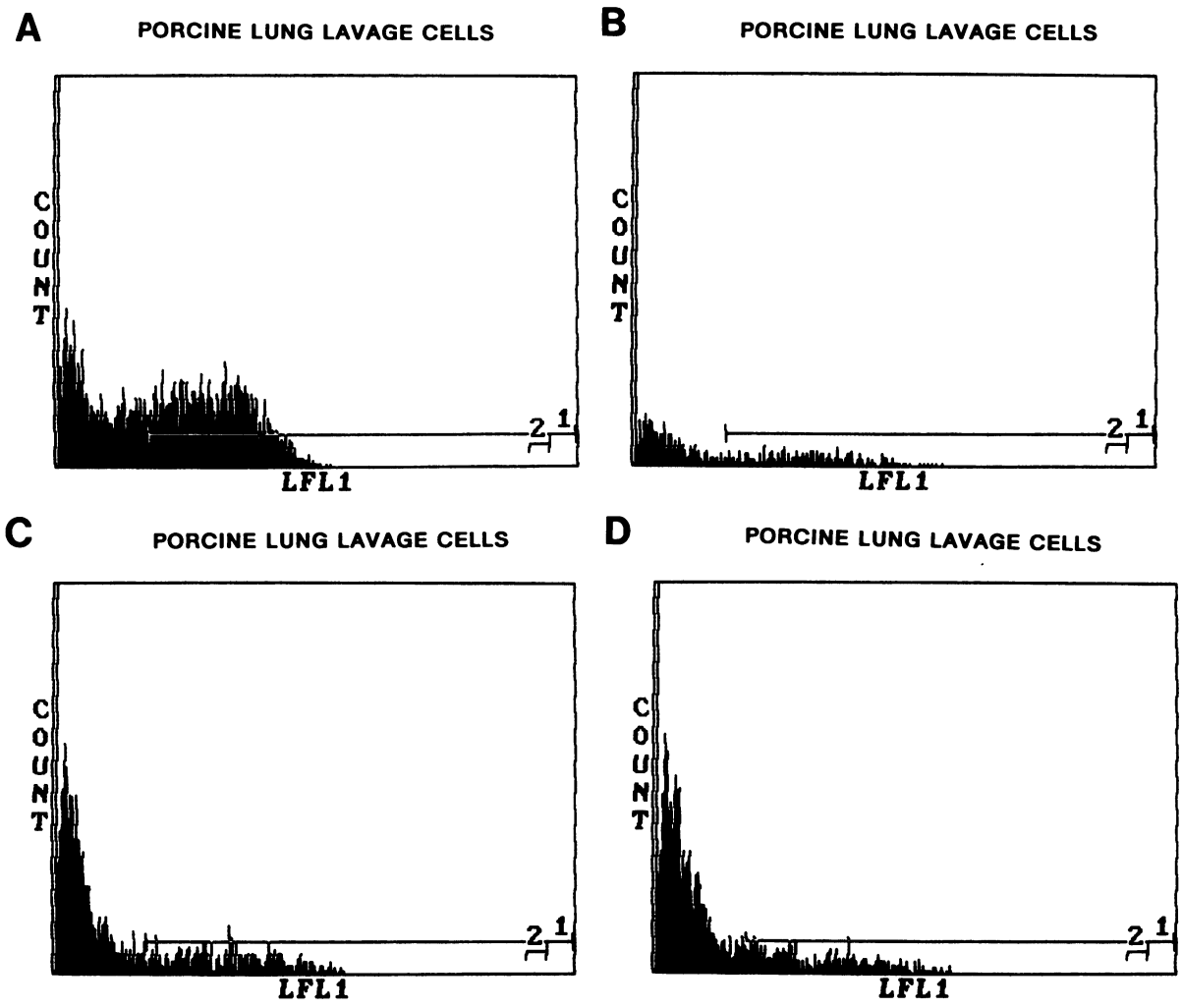

Figure 1. *Cytofluorographic analysis of porcine lung cells using A2C. Pulmonary lavage cells were obtained from pigs by exhaustive instillation and removal of normal saline. The cells were treated in suspension with* (A) *normal rabbit serum, followed by A2C, followed by FITC-rabbit anti-rat IgG;* (B) *normal rabbit serum, followed by normal rat serum, followed by FITC rabbit anti-rat IgG;* (C) *normal rabbit serum, followed by a mixture of rat monoclonal antibodies to Curosurf, followed by FITC-rabbit anti-rat IgG; and* (D) *normal rabbit serum, followed by FITC-rabbit anti-rat IgG. Cells were fixed in PBS-1% formalin, and analyzed by cytofluorography (Becton-Dickinson, FACS II). The ordinate represents the cell number, while the abscissa represents the log of the fluorescence intensity, with a channel range of 1 to 1000. The intense staining of large numbers of cells using A2C represents the only significant deviation from the background pattern seen in* (D).

from the opposite species. Thus A2R binds a 30-kd protein in both porcine and rabbit lungs, with some reactivity to proteins of 52 and 60 kd. Proteins of the same sizes are bound by A2C in both lung preparations (Figure 6).

Inhibition of Binding of SRF to Lung Cells

We determined whether anti-[anti-surfactant] antibodies altered the binding of radiolabeled SRFs to lung cell membranes. Viable (more than 95%) pulmonary lavage cells were obtained by centrifugation in a Ficoll gradient. A2C, A2R, or control antibody was added to these lung cells in suspension. After incubation, ^{125}I-labeled SRF was added and bound SRF counted. Preincubation with either of the anti-idiotypic antibody to porcine SRF receptor decreased binding of SRF to lung cells by 50% to 60% relative to controls (Tables 1,2). Preincubating rabbit lung cells with anti-idiotypic antibody to rabbit SRF receptor decreased binding by approximately 40% relative to controls. A comparable decrease was observed when 1000-fold excess of unlabeled surfactant was added to the alveolar wash cells. Pretreating (in this example) rabbit alveolar cells with saline or control culture supernatant resulted in considerably greater binding of radiolabeled rabbit SRF than did pretreatment of these lung cells with A2R, A2C, and unlabeled SRF ($P < 0.01$). In this example, A2C was as effective as A2R in inhibiting binding of iodinated SRF to rabbit lung cells. Both of these antibodies and excess unlabeled homologous SRF inhibited SRF binding to rabbit lung cells comparably.

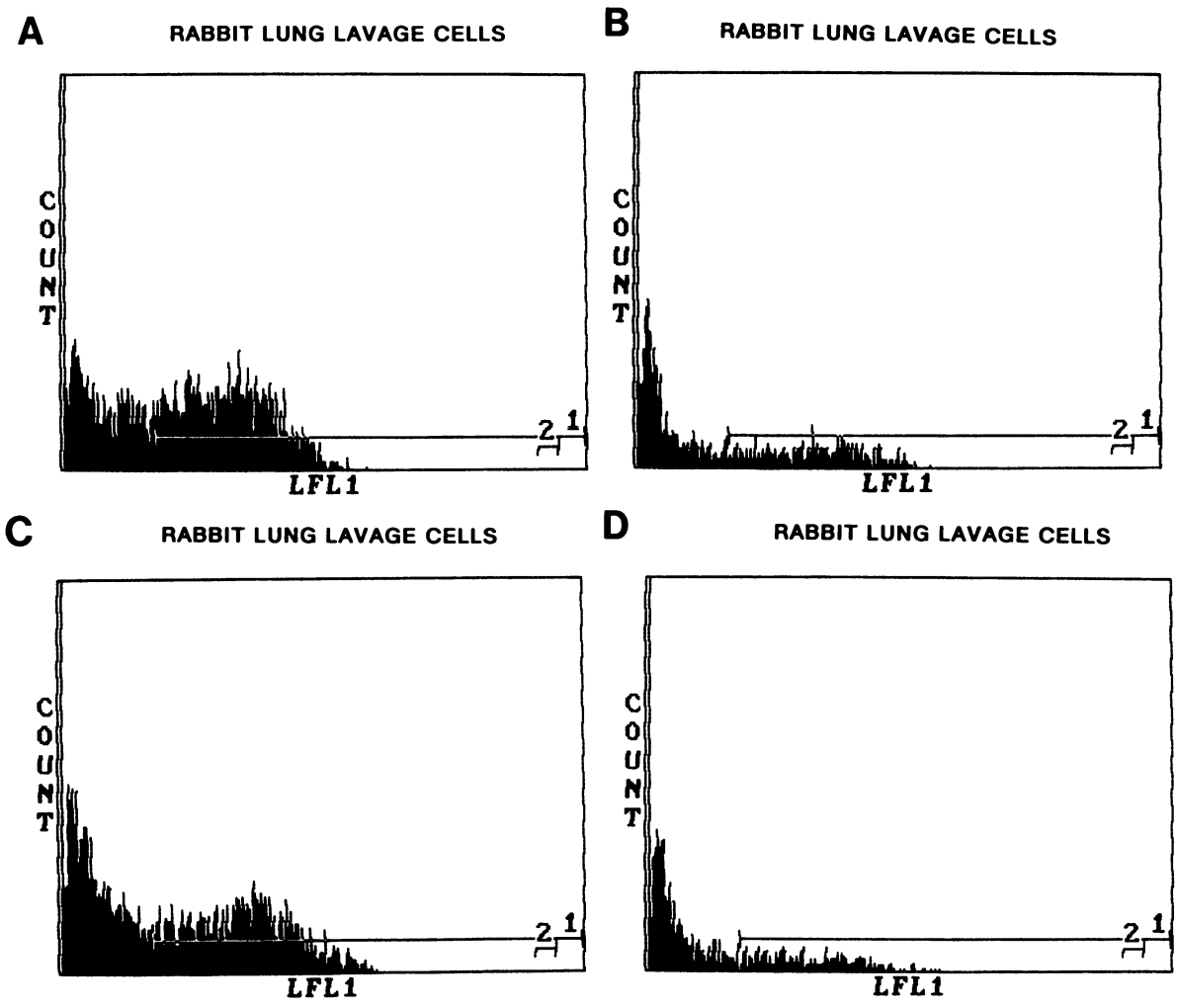

Figure 2. *Cytofluorographic analysis of porcine lung cells using A2R. Pulmonary lavage cells were obtained from pigs by exhaustive instillation and removal of normal saline. The cells were treated in suspension with* (A) *normal rabbit serum, followed by A2R, followed by FITC-rabbit anti-rat IgG;* (B) *normal rabbit serum, followed by normal rat serum, followed by FITC rabbit anti-rat IgG;* (C) *normal rabbit serum, followed by a mixture of rat monoclonal antibodies to Curosurf, followed by FITC-rabbit anti-rat IgG; and* (D) *normal rabbit serum, followed by FITC-rabbit anti-rat IgG. Cells then were fixed in PBS-1% formalin and analyzed by cytofluorography (Becton-Dickinson, FACS II). The ordinate represents the cell number, while the abscissa represents the log of the fluorescence intensity, with a channel range of 1 to 1000. The intense staining of large numbers of cells using A2R represents the only significant deviation from the background pattern seen in* (D).

Discussion

We report here two monoclonal anti-idiotype antibodies that recognize a lung cell membrane protein that in turn binds surfactant. These antibodies were developed by applying the principle that if two different molecules bind the same ligand their ligand-binding sites may have similar structures. At least some of the monoclonal antibodies that bind and inactivate proteins in porcine and rabbit surfactant (probably SP-B) preparations are likely to recognize that protein similarly to the way SRF receptors on pulmonary cells recognize it. A second set of antibodies that in turn recognize the idiotypic (antigen-binding) region of anti-SRF antibodies should then also recognize a comparable SRF-binding site on cell membrane SRF receptor on pulmonary cells.

Use of anti-idiotype antibodies to identify specific cell membrane receptors is well described.[22–24] We have identified two monoclonal rat antibodies directed against rat anti-SRF MAbs. One anti-idiotype antibody (A2C) binds anti-Curosurf MAbs. The other anti-idiotype antibody (A2R) binds anti-rabbit SRF MAbs. Anti-[anti-SRF] MAbs bind ciliated columnar bronchiolar and bronchial lining epithelial cells and alveolar lining cells.

They appear to bind these cells because they recognize a 30-kd protein on their cell membranes. By binding to these cell membrane proteins, anti-[anti-SRF] reduce the ability of these cells to bind radiolabeled SRF. Furthermore pre-exposure of lung cells to SRF inhibits the ability of these antibodies to bind. From these analyses we cannot determine the type of alveolar cells bound by A2C. However we are using immunoelectron microscopy

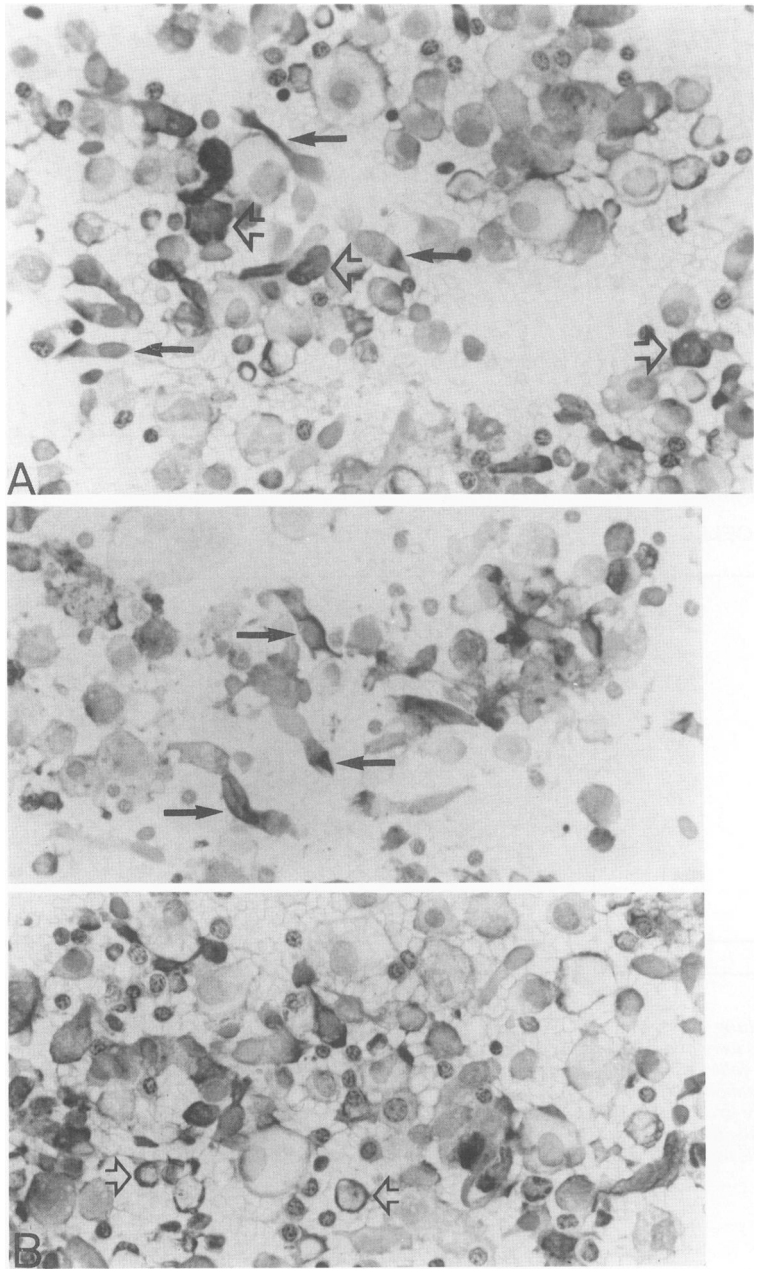

Figure 3. *Lung cells bound by A2C and A2R. Pulmonary lavage cells were stained with A2C* (A) *and A2R* (B) *as described in Materials and Methods. Open arrows show representative alveolar lining cells. Solid arrows show representative ciliated bronchial and bronchiolar cells. These are the two populations of cells positive for the protein recognized by the monoclonal anti-idiotype antibodies A2C and A2R. Large, unstained cells are macrophages. Sections treated with culture supernatant of YB2/0 cells not producing A2C were completely negative (not shown). Original magnification, ×860.*

to define better the specific alveolar lining cell population recognized by A2C and A2R.

This protein is the major species recognized by both A2C and A2R. Both MAbs also bind larger proteins at 52 and 60 kd in these preparations. Whether these larger proteins represent dimers of the smaller proteins, glycosylated forms, precursors, or partial degradation products of any of these, is not clear. Alternatively the SRF binding protein in question may be a heterodimer or heterotrimer, one of whose components is recognized by the A2C and A2R antibodies.

These monoclonal anti-idiotype antibodies also cross-react. A2C recognizes comparable proteins in rabbit lungs as in porcine lungs. A2R binds similar proteins in pig lungs as in rabbit lungs. Immunohistochemical analysis indicates that both antibodies bind the same cells in both animal species' lungs: alveolar lining cells and bronchial or bronchiolar epithelial cells.

We have performed comparable Western blot analyses of human lung proteins using these antibodies. In some experiments, A2C and A2R bind human lung proteins similar to those identified in porcine and rabbit

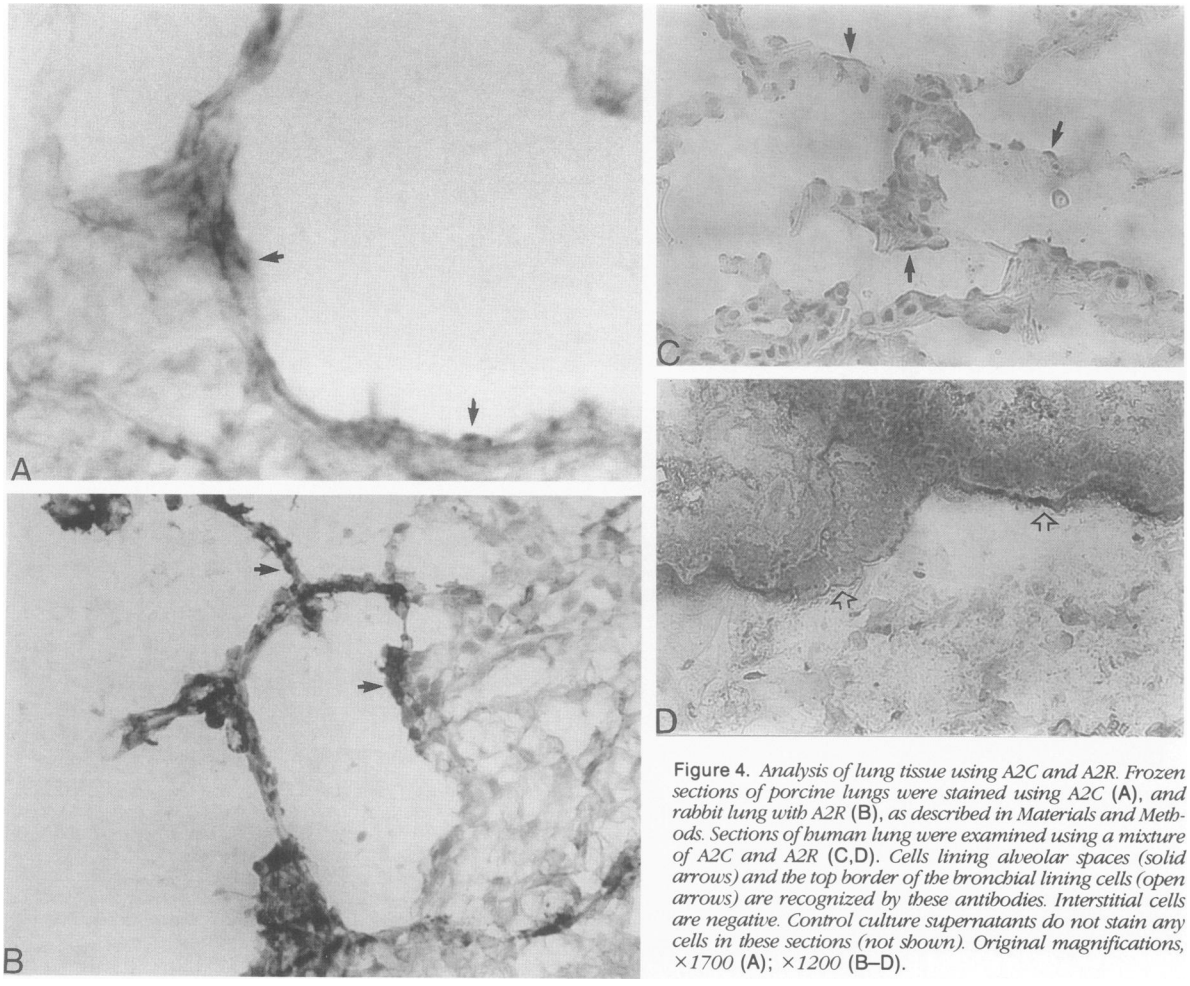

Figure 4. *Analysis of lung tissue using A2C and A2R. Frozen sections of porcine lungs were stained using A2C* (A), *and rabbit lung with A2R* (B), *as described in Materials and Methods. Sections of human lung were examined using a mixture of A2C and A2R* (C,D). *Cells lining alveolar spaces (solid arrows) and the top border of the bronchial lining cells (open arrows) are recognized by these antibodies. Interstitial cells are negative. Control culture supernatants do not stain any cells in these sections (not shown). Original magnifications, ×1700* (A); *×1200* (B–D).

lungs. However these results are inconsistent and so are not included here. (Strayer D, unpublished observations). Immunohistochemical analysis of human lung stained with A2C and A2R does not differ from observations made with rabbit and porcine lung.

In addition, we have used A2C and A2R to screen human and porcine lung cDNA expression libraries in λgt11 phage. Comparable cDNAs that encode proteins binding A2C and A2R have been identified in both libraries (Lee C, Strayer D, unpublished observations). Analysis of these clones is underway.

On the basis of these observations, it is likely that the 30-kd protein recognized by these monoclonal anti-idiotype antibodies is a specific cell membrane-binding protein for a component of surfactant. Whether this 30-kd protein mediates effects on specific intracellular or extracellular functions is not yet known. Receptor ligand binding may control a number of activities, eg, altered production of the ligand, activation of receptor-bearing cells, and extracellular handling and recirculation of the ligand. Whether this protein is truly a SRF receptor thus is not clear. However, in the more general sense that this protein binds SRF and is located on pulmonary cells where SRF binding, production, and recirculation occur, it may be considered a receptor of still indeterminate function. With the above functional caveats in mind, we shall refer to the 30-kd protein as a receptor for alveolar surfactant.

A2C and A2R display characteristics of anti-receptor antibodies: they were developed using as antigen a mixture of MAbs against proteins in defined SRF preparations whose protein constituents are only 4 kd and 9 kd (SP-C and SP-B in Curosurf) and 9 kd and 35 kd (SP-B and SP-A in RSRF); they bind cell membranes and recognize a specific protein on them; and they inhibit SRF binding to alveolar cells and SRF inhibits the ability of these antibodies to bind to alveolar cells. In addition, observations made using these two antibodies are mutually supportive: the similarity of the results obtained above using A2C and A2R raised from rats immunized with mixtures of anti-SRF antibodies to different animals' SRFs prepared differently and sharing only their respective SP-B. Our studies leave some ambiguity as to the nature of

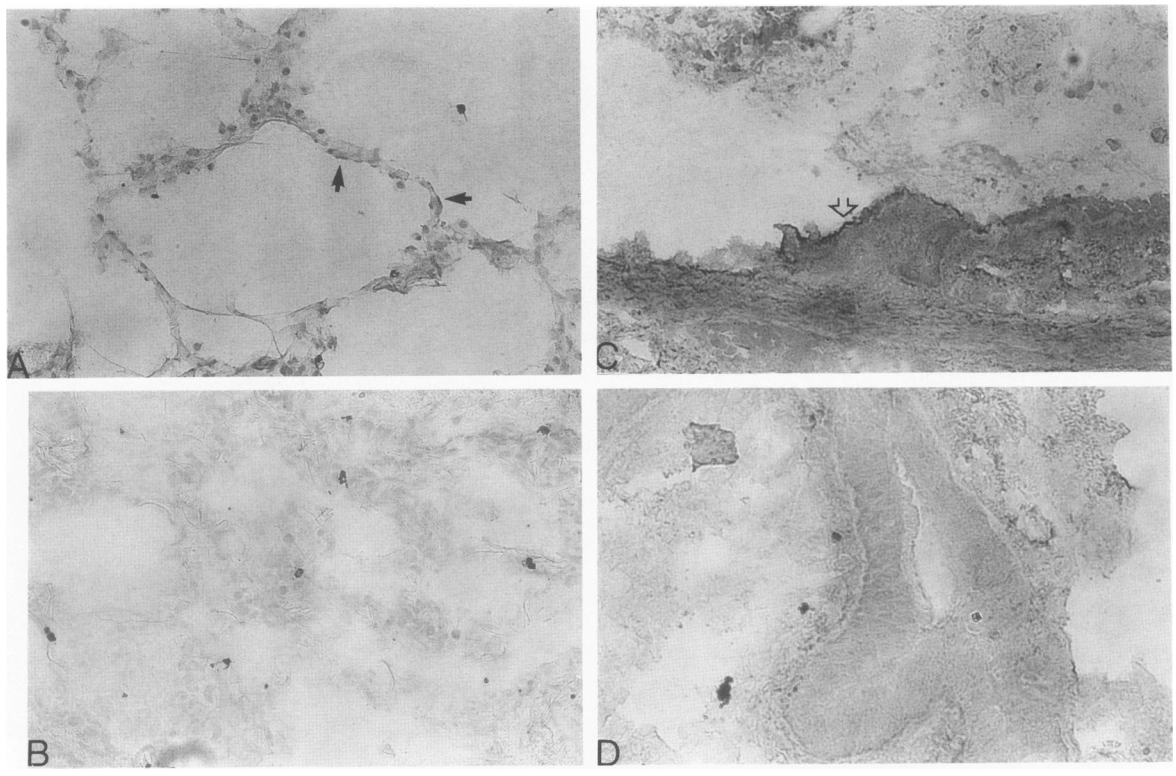

Figure 5. *Surfactant inhibition of A2R and A2C binding to lung tissue. Frozen sections of lung tissue* (A,B) *and bronchus* (C,D) *were preincubated with homologous SRF in saline* (A,C) *or saline* (B,D) *as described in Materials and Methods. They were then exposed to A2C and A2R and stained as before. Representative results are illustrated here. Preincubation with surfactant greatly diminishes the ability of anti-receptor antibodies to bind to alveolar lining cells* ([A] *as compared to* [B]) *and bronchial lining cells* ([C] *as compared to* [D]). *Positive staining is represented by cells highlighted by solid (alveolar lining cells) or open (bronchial lining cells) arrows. To ascertain that the peroxidase reaction worked for the slides incubated with surfactant to inhibit A2C and A2R binding, we used the internal peroxidases as internal positive controls: slides were not preincubated with* H_2O_2. *Mast cells and eosinophils therefore are noted to react positively because of their endogenous peroxidases* (B,D). *Original magnification,* ×1200.

the SRF protein that is the ligand for this receptor. We hope studies in progress using purified porcine SRF components will clarify this question.

The metabolism of surfactant by the lung has been a subject of intense investigation in recent years. A number of investigators have hypothesized that SRF is principally metabolized within the lungs,[25] that type II cells recognize SRF proteins, and that these cells are responsible for SRF recycling.[9,10] Our results support this theory, suggesting that a specific receptor for a SRF species exists and that it can be detected by a monoclonal antibody. The two monoclonal antibodies should be a useful tool in the study of SRF metabolism.

The reactivity of A2C and A2R, both with bronchial and bronchiolar epithelial cells as well as with alveolar lining cells, suggests that the former may represent an additional cell population that has heretofore not received much attention as regards SRF binding and/or recycling. Other investigators have found recently that there is a substantial concentration of SRF in the ciliary heads of these cells and suggest that the concentration of SRF there may be important in clearance functions (Schürch S, oral personal communication, June 1990).

Pettenazzo and coworkers[25] found that in rabbits very little (less than 5%) SRF, measured as ^{3}H-dipalmitoyl-phosphatidyl choline, travels the distance of the upper airways to reach the digestive tract. For more than 95% of SRF to be captured in alveoli would require highly efficient recognition by alveolar lining cells. Participation of bronchial and bronchiolar lining cells in this process would enhance the efficiency of the recycling/removal process because the surface area of these cells is great. Surfactant proteins that do not bind to alveolar lining cells thus may be prevented from leaving the respiratory tract by cells lining the larger airways. The subsequent fate of protein binding to these columnar cells remains to be determined.

Most studies of SRF protein metabolism and fate have concentrated on SP-A. One recent report localizes reactivity with antibody to human SP-B to alveolar lining cells.[26] Curosurf contains no detectable SP-A. The 30-kd cell membrane protein we describe here thus may relate

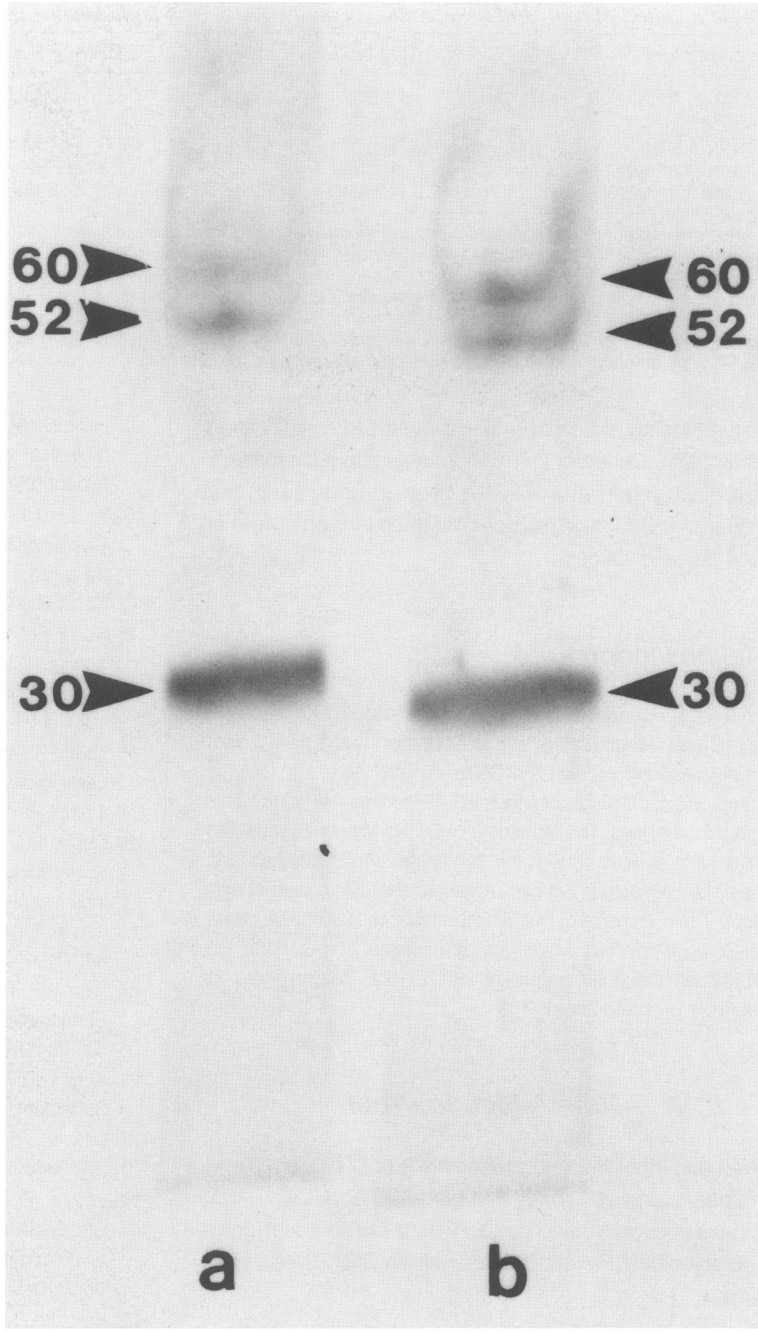

Figure 6. *Western blot of proteins from pulmonary cells. Crude cell lysates from lung lavage cells were prepared as described in Materials and Methods. Lysates were electrophoresed in SDS-10% PAGE and transferred to Immobilon. Preparations from pig lungs were exposed to A2C* (A) *and those from rabbit lungs to A2R* (B)*, followed by rabbit anti-rat IgG and then by* ^{125}*I-*Staphyloccocus *protein A. Blots were visualized by autoradiography. The molecular weight of the protein is indicated at the left and was inferred by interpolating from coelectrophoresis of standards of known molecular weight.*

Table 1. *Effect of Anti-[anti-Curosurf] Antibodies on the Binding of Radiolabeled Surfactant to Porcine Lung Cells*

Pretreatment	Cell type	CPM ^{125}I-SRF bound	Significance*
A2C	Pig	4706 ± 255	—
Control culture	Pig	10,578 ± 725	$P = 0.0006$
Normal saline	Pig	11,186 ± 1150	$P = 0.0014$

Normal porcine pulmonary lavage cells were treated with monoclonal anti-[anti-Curosurf] as culture supernatant in Roswell Park Memorial Institute medium 1640 supplemented with 10% horse serum, control culture supernatant (YB2/0 supernatant), or saline. Subsequently ^{125}I-labeled Curosurf was added to these cells and incubated. After washing, cells were counted to quantitate bound radioactive SRF. Data shown are means ± SEM of six independent determinations in each group.

* Statistical significance as determined by paired, two-tailed *t*-test of either YB2/0 supernatant before treatment *versus* A2C before treatment or normal saline *versus* A2C before treatment.

Table 2. *Effect of Anti-[anti-RSRF] Antibody on the Binding of Radiolabeled RSRF to Rabbit Lung Cells*

Pretreatment	Cell type	CPM ^{125}I-SRF bound	Significance*
A2R	Rabbit	8033 ± 807	—
A2C	Rabbit	7261 ± 650	$P = 0.07$
RSRF (4 μg)	Rabbit	8199 ± 272	$P = 0.83$
Control culture	Rabbit	12,792 ± 40	$P = 0.009$
Normal saline	Rabbit	12,603 ± 73	$P = 0.01$

Normal rabbit lung lavage cells were pretreated with monoclonal anti-[anti-rabbit SRF] as culture supernatant in Roswell Park Memorial Institute medium 1640 supplemented with 10% horse serum, approximately 1000-fold excess of unlabeled rabbit surfactant, anti-[anti-Curosurf], control supernatant (YB2/0 supernatant), or saline. Subsequently ^{125}I-labeled rabbit SRF was added to these cells and incubated. After washing, cells were counted to quantitate bound radioactive SRF. Data shown are the means ± SEM of three independent determinations in each group.

* Statistical significance as determined by paired, two-tailed *t*-test of either the condition in question *versus* A2R before treatment. A2C and SRF before treatment were not significantly different from A2R before treatment or from each other. A2R, A2C, and SRF pretreatment were all significantly different from either control medium or saline before treatment.

to the binding of a smaller SRF protein. Our identification of a putative receptor for a SRF protein suggests that this approach might profitably be used to understand the generation and maintenance of surface tension in normal and disease states.

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Note Added in Proof

We have identified and characterized cDNA clones in a human expression library that bind A2C and A2R. These clones encode a 30-kd protein that, to our surprise, binds recombinant SP-A protein as well as SRF protein mixtures.

References

1. Possmayer F: A proposed nomenclature for pulmonary surfactant-associated proteins. Am Rev Respir Dis 1988, 138:990–998
2. Wright JR, Clements JA: Metabolism and turnover of lung surfactant. Am Rev Respir Dis 1987, 135:426–444
3. Hawgood S, Benson BJ, Hamilton RL: Effects of a surfactant-associated protein and calcium ions on the structure and surface activity of lung surfactant lipids. Biochemistry 1985, 24:184–190
4. Revak SD, Merritt TA, Degryse E, Stefani L, Courtney M, Hallman M, Cochrane CG: Use of human surfactant low molecular weight apoproteins in the re-constitution of surfactant biologic activity. J Clin Invest 1988, 81:826–33
5. Yu S-H, Chung W, Olafson RW, Harding PGR, Possmayer F: Characterization of the small hydrophobic proteins associated with pulmonary surfactant. Biochim Biophys Acta 1987, 921:437–448
6. Notter RH, Shapiro DL, Ohning B, Whitsett JA: Biophysical activity of synthetic phospholipids combined with purified lung surfactant 6,000 dalton apoprotein. Chem Phys Lipids 1987, 44:1–17
7. Young SL, Wright JR, Clements JA: Cellular uptake and processing of surfactant lipids and apoprotein SP-A by rat lung. J Appl Physiol 1989, 66:1336–1342
8. Dobbs LG, Wright JR, Hawgood S, Gonzalez R, Venstrom K, Nellenbogen J: Pulmonary surfactant and its components inhibit secretion of phosphatidylcholine from cultured rat alveolar type II cells. Proc Natl Acad Sci (USA) 1987, 84:1010–1014
9. Rice WR, Ross GF, Singleton FM, Dingle S, Whitsett JA: Surfactant-associated protein inhibits phospholipid secretion from type II cells. J Appl Physiol 1987, 63:692–698
10. Wright JR, Wager RE, Hawgood S, Dobbs L, Clements JA: Surfactant apoprotein M_r = 26,000-26,000 enhances uptake of liposomes by type II cells. J Biol Chem 1987, 262:2888–2894
11. Wright JR, Borchelt JD, Hawgood S: Lung surfactant apoprotein SP-A (26–36 kDa) binds with high affinity to isolated alveolar type II cells. Proc Natl Acad Sci (USA) 1989, 86:5410–5414
12. Kuroki K, Mason RJ, Voelker DR: Alveolar type II cells express a high-affinity receptor for pulmonary surfactant protein A. Proc Natl Acad Sci (USA) 1988, 85:5566–5570
13. Curstedt T, Jörnvall H, Robertson B, Bergman T, Berggren P: Two hydrophobic low-molecular-mass protein fractions of pulmonary surfactant. Eur J Biochem 1987, 168:255–262
14. Suzuki Y, Curstedt T, Grossmann G, Kobayashi T, Nilsson R, Nohara K, Robertson B: The role of the low molecular weight (≤ 15 000 daltons) apoproteins of pulmonary surfactant. Eur J Respir Dis 1986, 69:336–345
15. Hallman M, Merritt TA, Schneider H: Isolation of human surfactant from amniotic fluid and a pilot study of its efficacy in respiratory distress syndrome. Pediatrics 1983, 71:473–482
16. Talmadge DW, Claman HN: External labeling of proteins

with I^{131}, I^{125}, and I^{130}. In Williams CA, Chase MW, eds. Methods in Immunology and Immunochemistry, New York, Academic Press, 1967, pp 389–391

17. Strayer DS, Hallman M, Merritt TA: Immunogenicity of surfactant. I. Human alveolar surfactant. Clin Exp Immunol 1991;83:35–40
18. Strayer DS, Hallman M, Merritt TA: Immunogenicity of surfactant II. Porcine and bovine surfactants. Clin Exp Immunol 1991;83:41–46
19. Strayer DS, Leibowitz JL: Virus-lymphocyte interactions during the course of immunosuppressive virus infection. J Gen Virol 1987, 68:463–472
20. Hsu SM, Raine L, Fanger H: Use of avidin-biotin-peroxidase complex (ABC) in immunoperoxidase techniques: A comparison between ABC and unlabeled antibody (PAP) procedures. J Histochem Cytochem 1981, 29:577–580
21. Towbin H, Staheiln T, Gordon J: Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: Procedure and some applications. Proc Natl Acad Sci (USA) 1979, 76:4350–4354
22. Greene MI, Co MS: Shared structural features of an internal image bearing monoclonal anti-idiotype and the external antigen. Monogr Allergy 1987, 22:120–125
23. Greene MI, Kokai Y, Gaulton GN, Powell MB, Geller H, Cohen JA: Receptor systems in tissues of the nervous system. Immunol Rev 1987, 100:153–184
24. Strayer DS, Vitetta ES, Kohler H: Anti-receptor antibody I. Isolation and characterization of the immunoglobulin receptor for phosphorylcholine. J Immunol 1975, 114:722–727
25. Pettenazzo A, Jobe A, Humme J, Seidner S, Ikegami M: Clearance of surfactant phosphatidylcholine via the upper airways in rabbits. J Appl Physiol 1988, 65:2151–2155
26. Phelps DS, Harding HP: Immunohistochemical localization of a low molecular weight surfactant-associated protein in human lung. J Histochem Cytochem 1987, 35:1339–1342