Increase of C-Reactive Protein and Decrease of Surfactant Protein A in Surfactant after Lung Transplantation

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In this study, we asked whether the serum acute-phase protein C-reactive protein (CRP) increased in large surfactant aggregates after lung transplantation and analyzed the changes in composition and interfacial adsorption activity of those aggregates. Single left lung transplantation was performed in weight-matched pairs of dogs. A double-lung block from the donor animal was flushed with either modified Euro-Collins solution (EC) (n = 6) or University of Wisconsin solution (UW) (n = 6) at 4° C followed by immersion in cold EC or UW for 22 h. The left donor lung was transplanted. The recipient dog was then reperfused for 4.5 h. Irrespective of the preservation fluid, gas exchanged was impaired in the transplanted lung after 4.5 h of reperfusion. Large surfactant aggregates obtained from this lung showed reduced ability to rapidly adsorb to an air-liquid interface. Phospholipid (PL) content and PL composition of surfactant from lung transplants was similar to that of the control lungs. However, the content of surfactant protein A decreased after reperfusion. In addition, Western blot analyses showed that levels of CRP increased in surfactant from transplanted but not from donor lungs. The addition of human CRP to control surfactant (CRP:PL weight ratio, 0.01:1) caused a decrease of surfactant adsorption. We conclude that the impairment of adsorption facilities of surfactant from transplanted lungs may be correlated with decreased levels of surfactant protein A and increased levels of CRP. The presence of elevated levels of CRP in bronchoalveolar lavage could be a very sensitive marker of lung injury. Casals C, Varela A, Ruano MLF, Valiño F, Pérez-Gil J, Torre N, Jorge E, Tendillo F, Castillo-Olivares JL. Increase of C-reactive protein and decrease of surfactant protein A in surfactant after lung transplantation. AM J RESPIR CRIT CARE MED 1998;157:43–49.

Lung transplantation has become an option for selected patients with end-stage obstructive or infective lung disease. However, progress with lung transplantation lagged behind other organs, such as kidney, liver, and heart, in part because of problems unique to the lung (1). The lung is a delicate organ that can develop significant dysfunction in response to minor injury. The severe ischemia-reperfusion injury associated with lung transplantation is similar to the impairment found with acute respiratory distress syndrome (ARDS) (1, 2). In both cases, inflammatory mediators are believed to play a significant role in the cascade of events leading to lung dysfunction (1, 2). The alteration of alveolar surfactant system is an important factor contributing to lung dysfunction in ARDS (2–4) and after lung transplantation during the early reperfusion period (5, 6). In normal lung, alveolar surfactant serves to stabilize the alveoli and distal airways at low lung volumes (7). To fulfill this function, surfactant is composed of a complex mixture consisting of lipids (mainly phospholipids) (8) and three surfactant apolipoproteins (SP-A, SP-B, and SP-C) (9). Alteration in alveolar surfactant leads to a decrease in compliance, ventilation-perfusion mismatch including shunt flow due to altered gas flow distribution, and lung edema formation (2). The possible mechanisms of surfactant alterations in ARDS and maybe in lung transplants include (*1*) damage or inhibition of surfactant compounds by inflammatory mediators (proteases, oxygen free radicals, phospholipases, cytokines, or lipid mediators) (10, 11), (*2*) inhibition of the biophysical activity of surfactant by plasma protein leakage (12), and (*3*) reduced or altered generation of surfactant-active compounds (2, 13, 14).

Little is published on the changes in lung surfactant after lung transplantation. Recent studies (5, 6) indicated that the biophysical activity of alveolar surfactant was impaired during the early reperfusion period in part as a consequence of the inhibition of surfactant by serum proteins that leaked in large amounts into alveolar fluid. The purpose of this study was to

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characterize the changes in the composition and activity of pulmonary surfactant isolated from canine transplanted lungs and to investigate the presence of the acute-phase protein C-reactive protein (CRP) in surfactant from donor and transplanted lungs. CRP is produced predominantly by hepatocytes after infection or inflammation and secreted into the serum. In addition, alveolar macrophages can also produce and secrete CRP to the alveolar space (15). The CRP mRNA levels in isolated macrophages are up-regulated by *in vitro* lipopolysaccharide stimulation. This protein is a potent inhibitor of surfactant adsorption (16) and has a high calcium-dependent affinity for phosphocholine (17), which is the headgroup of the most abundant component of pulmonary surfactant, phosphatidylcholine.

METHODS

Experimental Groups

Single left lung transplantation procedures were performed in weight-matched pairs of dogs (20 to 25 kg) as described previously (18). A total of 24 dogs were randomly divided into two groups (n = 12 per group; 6 donors and 6 recipients): Group 1, lung flushing via the pulmonary artery with modified Euro-Collins (EC) solution at 4° C, followed by immersion in cold EC solution for 22 h; and Group 2, same procedure as in Group 1, but University of Wisconsin (UW) solution was used as the preservation solution. Animals (donors and recipients) were sedated with acepromazine (0.1 mg/kg body wt) followed by anesthetic induction with 2.5% thiopental sodium (6 mg/kg body wt) and maintenance with 1.5% isoflurane. All animals received human care in compliance with the Spanish regulations and law and in accordance with the "Principles of Laboratory Animal Care" (formulated by the Institute of Laboratory Animal Resources) and the "Guide for the Care and Use of Laboratory Animals" (NIH Publication No. 85-23, revised in 1985).

Donor Operation

The donor chest was opened by median-sternotomy, and ligatures were placed around both venae cavae. After heparin (3 mg/kg body wt) was administered intravenously, a single bolus of 1 ml (500 μg) prostaglandin E_1 diluted in 10 ml of saline was injected into the right ventricle outflow tract. When blood pressure dropped, inflow occlusion and cross-clamping of the aorta followed, and cardioplegic (crystalloid) and pulmoplegic solutions (EC or UW) were rapidly instillated. The lung preservation solution (60 ml/kg body wt) was administered from a bag raised to 30 cm above table level, and continuous ventilation was maintained while both pleural cavities were opened and copiously irrigated with cold saline.

The heart was removed, and the double-lung block excised. Both lungs were immersed in EC or UW solution at 4° C for 22 h until implantation. Afterwards, the donor right lung was lavaged to obtain the bronchoalveolar fluid (BAL), and the donor left lung was transplanted into the recipient animal.

Recipient Operation

Weight-matched recipients underwent a left posterolateral thoracotomy through the fifth intercostal space. The hemiazygos vein was ligated and divided, and the pericardium was opened anterior to the pulmonary veins. The three anastomotic stumps were prepared after clamping of the left pulmonary artery at the point of its bifurcation and the left main bronchus proximal to the last branch. Lastly, the left atrial appendage was isolated with a side-biting clamp. The left lung removed from recipient dogs was used as control lung to obtain control surfactant.

The donor left lung was anastomosed to the recipient. The airway anastomosis was performed with a single 4-0 prolene suture, and each vascular anastomosis was done with a single 5-0 suture. The pulmonary artery clamp was removed first to allow for backflow and dealing of the graft through the venous anastomosis. Ventilation was resumed at a rate of 17 ± 3 breaths/min (20 ml/kg body wt) and a positive end-expiratory pressure of +5 cm H_2O.

Four hours after the implantation procedure was complete, the right main bronchus and right pulmonary artery were cross-clamped for 30 min for the assessment of graft function on the basis of arterial blood gases. The right main bronchus and right pulmonary artery branch were previously dissected from the left pleural cavity. This was done prior to the left pneumonectomy.

BAL Processing and Isolation of Pulmonary Surfactant

After 4.5 h of reperfusion period, the transplanted lung was lavaged twice with 4° C saline (50 ml/kg body wt). BALs were pooled and immediately centrifuged at 400 × *g* for 10 min at 4° C to remove cells and cell debris. The volume of the remaining cell-free BAL was recorded, and an aliquot was taken for protein determination. BAL was stored at −20° C until used for surfactant isolation.

Large surfactant aggregates (heavy subtype surfactant) were obtained as previously described (13). Briefly, BAL was centrifuged at 100,000 × *g* for 2 h at 4° C to obtain the large surfactant aggregates in the resulting pellet. Because of the amount of leaked serum protein in BAL of transplanted lungs, measured by the Lowry method, large surfactant aggregates obtained by sedimentation were contaminated by lipids and proteins from blood transudated into the air spaces. To separate the surfactant from blood components, we used the method of Shelley and colleagues (19). Large surfactant aggregates from control, donor and transplanted lungs were resuspended in 16% NaBr (density: 1.14) and placed on the bottom of a tube, overlayered with 13% NaBr (density: 1.11) in 0.9% NaCl and then with saline containing no NaBr. The gradient was centrifuged at 116,000 × *g* for 2 h at 4° C. Surfactant has a density of about 1.085 at 4° C, which is lower than that of most of the contaminating components of serum. We previously showed (13) that with this procedure, surfactant from rabbit lungs with ARDS contained less serum components (sphingomyelin and proteins) and more surfactant phospholipids (phosphatidylglycerol) than the corresponding BAL of those pathologic lungs.

Large surfactant aggregates were analyzed for: (*1*) phospholipid and protein concentration, (*2*) content of SP-A and CRP, and (*3*) interfacial adsorption activity. In addition, lipid extracts of surfactants (LES) were prepared by chloroform/methanol extraction (13). LES were examined for: (*1*) phospholipid yield and composition and (*2*) SP-B and SP-C quantitation.

Surfactant Phospholipid Analysis

Total phospholipid was determined from aliquots of both surfactant and LES by phosphorus analysis as described by Rouser and associates (20). Phospholipid classes were separated by two-dimensional chromatography on precoated activated silica gel type 60 G thin-layer plates. The plates were developed in chloroform/methanol/H_2O (65:25:4, vol/vol) and methylacetate/*n*-propanol/chloroform/methanol/0.25% KCl (25:25:25:10:9, vol/vol), respectively. Lipid areas of chromatograms were visually detected by exposure to I_2 vapor. The spots corresponding to individual phospholipids were scraped off for phosphorus determination.

Quantitation of SP-B and SP-C

SP-B and SP-C were quantitated from LES by quantitative amino acid analysis as reported elsewhere (21). Aliquots of known phospholipid concentration of LES were dried under N_2. Then, hydrolysis of SP-B and SP-C was performed by adding 200 μl of 6 M HCl containing 0.1% (wt/vol) phenol and incubating at 108° C for 24 h in tubes sealed under vacuum. Norleucine was added to each sample as internal standard. Analyses were carried out on a Beckman System 6300 high-performance amino acid analyzer.

SP-B was quantitated from the chromatogram by using the detected amounts of Tyr, Glx, Thr, Asx, and His and the reported amino acid composition for canine SP-B (22). These amino acids are present in the composition of SP-B (22) but not in that of canine SP-C (23). SP-C was quantitated from the detected amounts of Phe. This amino acid is present in the amino acid composition of canine SP-C but not in that of canine SP-B. In addition, we considered the detected amounts of Pro, Gly, Ala, Met, Lys, and Arg in the chromatogram to quantify both SP-B and SP-C. These amino acids are present in the composition of both proteins in dogs. Thus, for SP-C quantiation, we discount to the total nanomoles of each of those amino acids, the nanomoles

calculated for each of them in the composition of SP-B. The same procedure was performed for SP-B quantitation from the mentioned amino acids.

The quantitation of SP-B and SP-C from LES by this method is reproducible. This method was validated by determining known amounts of SP-B and SP-C from mixtures prepared from these proteins previously isolated by chromatographic separation on Sephadex LH-60 and quantified by amino acid analysis (21). On the other hand, the amino acid composition of surfactant hydrophobic proteins analyzed in LES from transplanted lungs (EC or UW) was similar to that found in control lungs. This suggests that LES from transplanted lung is free of possible hydrophobic fragments derived upon enzymatic cleavage of plasma proteins.

Isolation of Canine SP-A

Canine SP-A was isolated from large surfactant aggregates of control lungs using sequential butanol and octyl glucoside extractions (24). SP-A concentrations were estimated by quantitative amino acid analysis as described above. The protein was stored in small aliquots in 5 mM Tris/HCl buffer, pH 7.4 at −20° C. Electrophoretic analysis of SP-A was performed under reducing conditions (50 mM dithiothreitol) by one-dimensional sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) using stacking and running gels of 4 and 12% acrylamide, respectively. Gels were stained with Coomassie Brilliant Blue R-250.

Measurement of SP-A and CRP in Surfactant

Electrophoretic analysis of surfactant from control and transplanted lungs (EC and UW) were performed as described above. Applied was either the same protein amount (15 μg protein) or the same phospholipid amount (200 nmol phospholipid) from all of the surfactant samples. In addition, 8 μg of canine SP-A was always applied in each gel. After electrophoresis, samples were transferred to nitrocellulose using a Bio-Rad Trans-Blot Cell. Transfer was carried out at 100 V constant voltage and 100 mA total increment of intensity, using 25 mM Tris (pH 8.3), 192 mM glycine, 20% (vol/vol) methanol as a transfer buffer. After drying, the nitrocellulose was blocked for 15 min with 10% dried skim milk in PBS-T (137 mM NaCl, 10 mM Na_2HPO_4, 0.18 mM KH_2PO_4, 2.7 mM KCl [pH 7.4], and 0.1% [vol/vol] Tween 20) and washed five times with PBS-T. Then, the primary antibody (anti-SP-A or anti-CRP) was added and incubated for 1 h in PBS-T. The blot was washed five times with PBS-T and incubated for 15 min with the secondary antibody, peroxidase-labeled anti-rabbit IgG antibody (Amersham International, Buckingshamshire, UK). After washing five times, detection reagents were added and SP-A or CRP observed on a Hyperfilm™ ECL. Quantification was finally achieved by densitometric evaluation in an Ultroscan 2002 densitometer (LKB, Uppsala, Sweden). For SP-A, an anti-(human-SP-A) polyclonal antibody, kindly supplied by Dr. J. A. Whitsett (University of Cincinnati, Cincinnati, OH), was used. For CRP, an anti-(rat-CRP) polyclonal antibody was generously gifted by Dr. S. Mookerjea (Memorial University of Newfoundland, St. John's Canada).

Adsorption Assay

The ability of surfactant, isolated from control and transplanted (EC or UW) lungs, to adsorb onto and spread at the air–water interface was tested at 25° C on a King-Clements apparatus consisting in a Wilhelmy-like high-sensitive surface microbalance, coupled to a Teflon™ dish of very small size (25). The samples were injected into the hypophase chamber of the Teflon dish which contained 5 ml of 5 mM Hepes buffer (pH 7.0), 150 mM NaCl, and 5 mM $CaCl_2$, with continuous stirring. The amount of surfactant phospholipids injected in the hypophase was 350 nmol for all of the samples. Interfacial adsorption was measured following the change in surface tension as a function of time. For each preparation, the analysis was repeated three times.

Statistical Analysis

All data represent the mean of individual measurements ± SD. Student's *t* test was used to analyze the difference between the means. Differences with a p value of < 0.05 were considered statistically significant.

TABLE 1

LUNG TRANSPLANT FUNCTION*

Step	Preservation Solution	P_{O_2} (*mm Hg*)	P_{CO_2} (*mm Hg*)
Baseline	EC	504 ± 36	44 ± 11
	UW	412 ± 55	40 ± 6
Post-surgery	EC	436 ± 47	50 ± 15
	UW	452 ± 40	42 ± 6
After 4 h of reperfusion†	EC	56 ± 24‡	75 ± 21
	UW	76 ± 40‡	49 ± 13

Definiton of abbreviations: EC = Euro-Collins solution; UW = University of Wisconsin solution.

* n = 6.

† Lung transplant function was assessed after 4 h of reperfusion by measuring arterial blood gases in the left pulmonary artery with the right pulmonary artery snared.

‡ $p < 0.01$ versus control.

RESULTS

Graft function after 4.5 h of reperfusion was assessed by measuring P_{O_2} later than the right main bronchus and right pulmonary artery were cross-clamped for 30-min (Table 1). A significant decrease of P_{O_2} was found. No significant difference was observed between EC and UW solutions.

Figure 1 shows an analysis of phospholipid content and protein/phospholipid ratio in large surfactant aggregates and in LES (hydrophobic protein/phospholipid). The content of phospholipids in surfactant from transplanted lungs was not significantly different from that of control lungs. However, a significant increase of the protein/phospholipid ratio was found in transplanted lungs preserved with either EC or UW solution despite the purification procedure used to separate surfactant from blood components transudated into the air spaces. The protein/phospholipid ratio of surfactant from transplanted versus control lungs was 1.47 ± 0.5 for the EC group and 1.48 ± 0.6 for the UW group. On the other hand, the hydrophobic protein/phospholipid ratio significantly decreased in transplanted lungs preserved with EC solution but not in those preserved with UW solution. Further phospholipid analysis of LES revealed a very similar phospholipid composition in control and transplanted (EC or UW) lungs (Figure 2).

In vitro surfactant function was determined by measuring the ability of surfactant to adsorb and spread at an air-water interface in a diffusion-independent system (Figure 3). As the interfacial adsorption rate is dependent on the concentration of surfactant material in the hypophase, the concentration of surfactant phospholipids was deliberately chosen so as to obtain a measurable rate of change of surface pressure (π) which was not instantaneous, to be sure that any change on the rate of surfactant adsorption could be detected under the experimental conditions. The amount of surfactant phospholipids injected into the hypophase was the same for all the samples from control and transplanted groups. Figure 3 shows that the adsorption rate decreased in surfactant from transplanted lungs. The results obtained with EC solution were not significantly different from those with UW solution.

Figure 4A shows electrophoretic analysis of pulmonary surfactant from control and transplanted lungs and Western blot analysis of SP-A. Serum protein contamination is easily visible by SDS-PAGE in partly purified large surfactant aggregates from lung transplants. The content of SP-A decreased by 57% in surfactant from transplanted lungs (Figure 4B). No significant difference was observed between the EC and UW groups. That marked decrease in the SP-A content

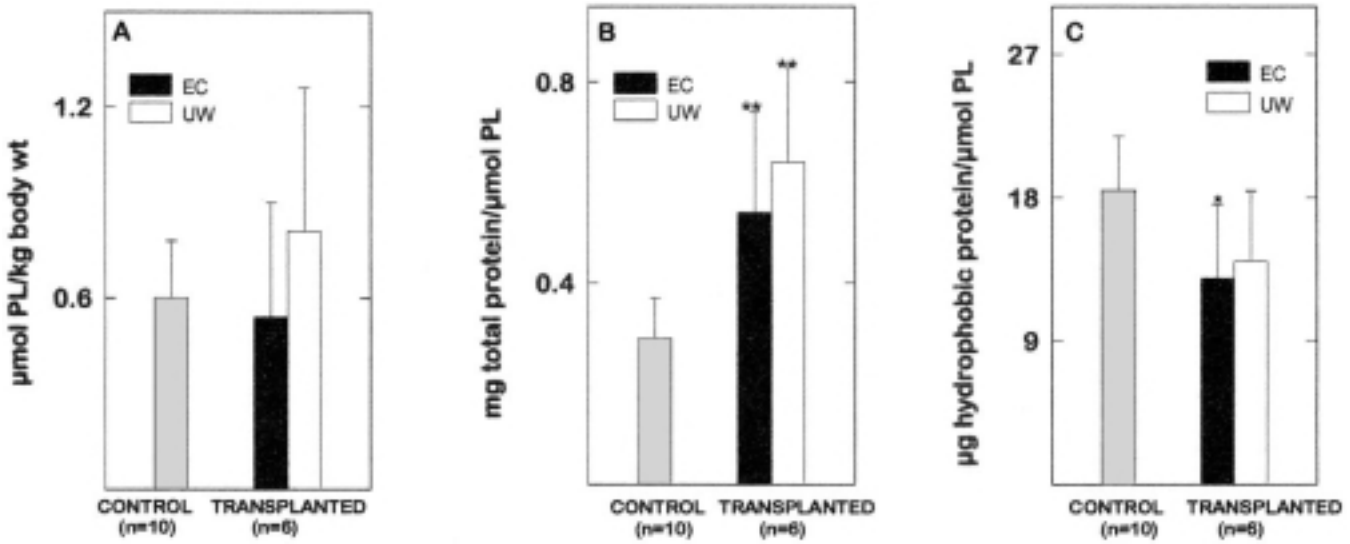

Figure 1. Phospholipid content and protein/phospholipid ratio in surfactant from control and transplanted lungs. Total protein was measured by the Lowry method. Hydrophobic protein was measured from lipid extract of surfactant (LES) by quantitative amino acid analysis. Phospholipid content was determined both in surfactant and in LES. EC = Euro-Collins solution; UW = University of Wisconsin solution. $^{**}p < 0.01$ and $^{*}p < 0.05$ versus control.

occurred after reperfusion because SP-A content did not decrease in lungs preserved with either EC or UW solution (data not shown). The content of SP-B and SP-C in a known amount of phospholipids was estimated by amino acid analysis of LES (Table 2). Irrespective of the preservation fluid, SP-B but not SP-C slightly decreased in surfactant from transplanted lungs.

Immunoblot analysis for CRP is shown in Figure 5. The content of CRP significantly increased in surfactant from transplanted lungs preserved with either EC or UW solution. Differences between the EC and UW group did not achieve statistical significance. The content of CRP did not change in surfactant from donor lungs flushed with EC or UW solution (data not shown). Thus, the increase of CRP content in surfactant from transplanted lungs occurred after reperfusion.

To find out if CRP might in part be responsible for the observed decrease in surfactant adsorption activity, we studied

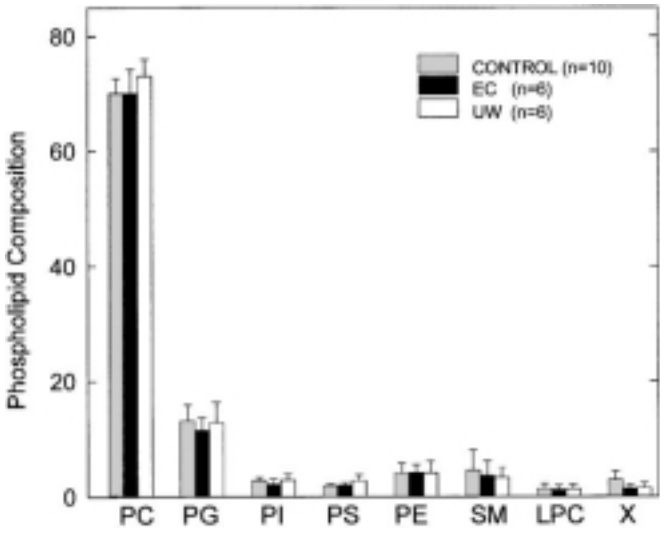

Figure 2. Phospholipid composition of surfactant from control and transplanted lungs. PC = phosphatidylcholine; PG = phosphatidylglycerol; PI = phosphatidylinositol; PS = phosphatidylserine; PE = phosphatidylethanolamine; SM = sphingomyelin; LPC = lysophosphatidylcholine; X = bismonoacylglycerol phosphate; EC = Euro-Collins solution; UW = University of Wisconsin solution.

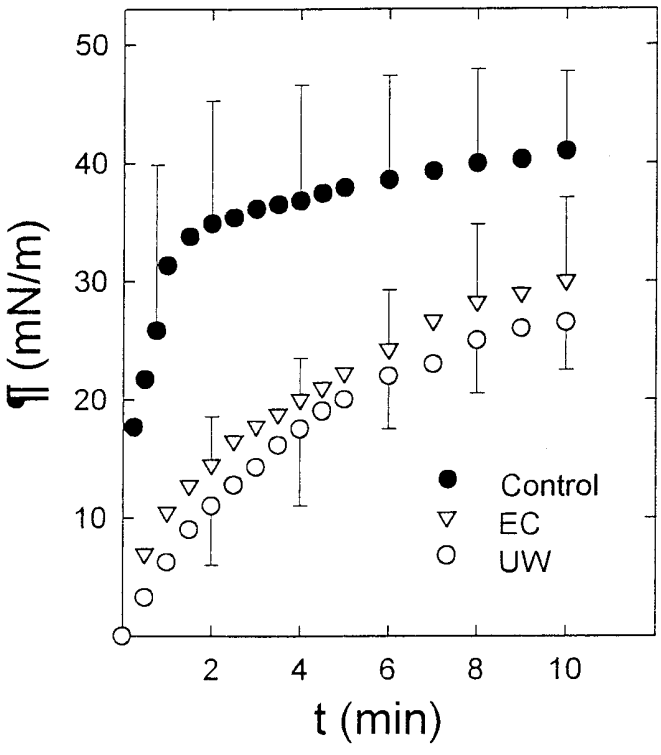

Figure 3. Interfacial adsorption kinetics of pulmonary surfactant isolated from control and transplanted lungs. π = surface pressure; EC = Euro-Collins solution; UW = University of Wisconsin solution. The final concentration of phospholipids in the hypophase was 59.8 nmol/ml for all surfactant preparations obtained from control (n = 6) and transplanted lungs (EC, n = 6; UW, n = 6). Values are expressed as mean ± SD.

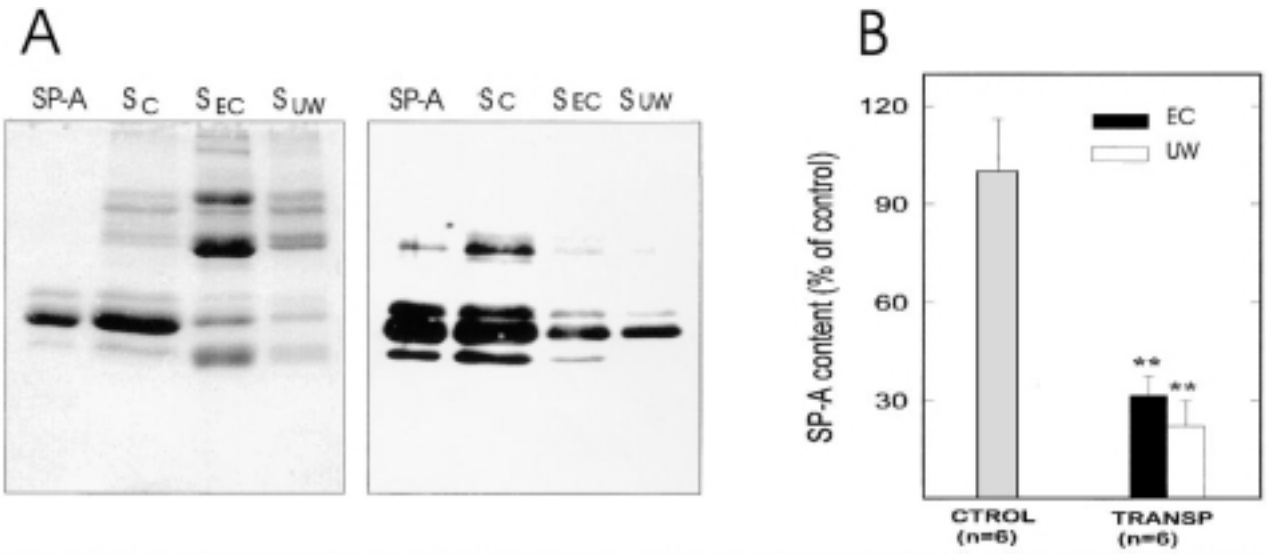

Figure 4. (*A*) Electrophoretic staining patterns of surfactant proteins separated under reducing conditions (*left panel*) and Western blot analysis for SP-A (*right panel*). SP-A = 8 μg of canine SP-A isolated from control lungs; S_c = 15 μg of surfactant proteins from control lungs; S_{EC} = 15 μg of surfactant proteins from transplanted lung preserved in Euro-Collins solution; S_{UW} = 15 μg of surfactant proteins from transplanted lung preserved in University of Wisconsin solution. Western blot was obtained by transferring proteins from a duplicate SDS-PAGE gel onto nitrocellulose and then blotting with anti-SP-A antibody as described in METHODS (*B*) SP-A content of surfactant from control and transplanted lungs. Quantitation of SP-A was achieved by densitometric evaluation of the 36 to 40 kDa SP-A bands obtained from SDS-PAGE under reducing conditions. A volume of surfactant corresponding to 200 nmol of phospholipids was applied for all of the surfactant preparations (control, n = 6; EC, n = 6; UW, n = 6). **$p < 0.01$ versus control.

the influence of human CRP (Calbiochem, La Jolla, CA) on the adsorption rate of control surfactant at various CRP/phospholipid weight ratios (Figure 6A). At a very low CRP/phospholipid weight ratio (0.01:1) (0.45 μg CRP/ml), surfactant adsorption rate was impaired. Higher CRP/phospholipid ratios caused higher inhibition of surfactant adsorption. The inhibition of surfactant adsorption by CRP was completely reversed by addition of phosphocholine. However, the reduced adsorption rate of surfactant from lung transplants was not improved by addition of excess phosphocholine (Figure 6B), indicating that other factors, such as the decrease of SP-A and SP-B levels and the presence of other serum proteins, must be also involved in the impairment of surfactant adsorption activity. Interestingly, addition of SP-A, in the absence or the presence of phosphocholine, was unable to reverse inhibition of the adsorption activity of surfactant from transplanted lungs.

DISCUSSION

Pulmonary edema associated with pulmonary hypertension and hypoxemia often occurs in early post-transplant period.

TABLE 2

SP-B AND SP-C CONTENT IN SURFACTANT FROM CONTROL AND TRANSPLANTED LUNGS*

Group	nmol SP-B†/μmol PL	nmol SP-C/μmol PL
Control	1.06 ± 0.2 (n = 10)	2.27 ± 0.4 (n = 10)
Transplanted (EC)	0.75 ± 0.3 (n = 6)‡	1.63 ± 0.5 (n = 6)
Transplanted (UW)	0.73 ± 0.2 (n = 6)‡	1.95 ± 0.6 (n = 6)

Definiton of abbreviations: SP-B = surfactant protein B; SP-C = surfactant protein C; PL = phospholipid; EC = Euro-Collins solution; UW = University of Wisconsin solution.

* SP-B and SP-C content was estimated by quantitative amino acid analysis as described in METHODS.

† Monomeric SP-B.

‡ $p < 0.05$ versus control.

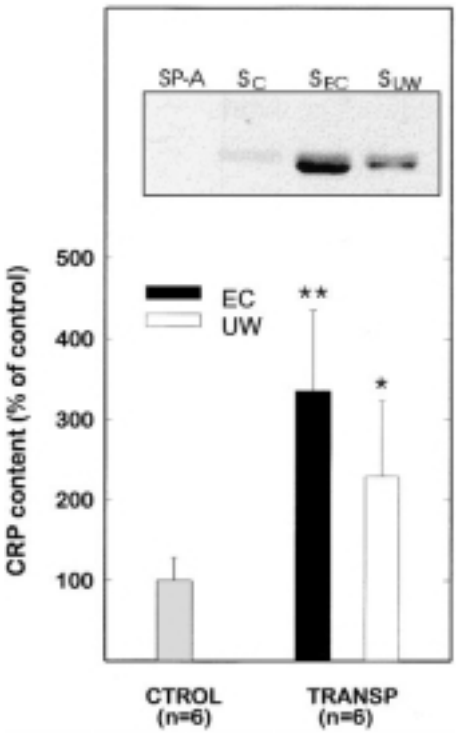

Figure 5. Levels of immunoreactive CRP in pulmonary surfactant isolated from control and transplanted lungs. Fifteen micrograms of surfactant proteins from control (n = 6) and transplanted lungs (EC, n = 6; EW, n = 6) were separated by gel electrophoresis, transferred to nitrocellulose, and blotted with polyclonal antibodies against CRP. Eight micrograms of canine SP-A were also loaded on the gels (*left lane*). Quantitation of CRP was achieved by densitometric evaluation of the immunoreactive bands. **$p < 0.01$ and *$p < 0.05$ versus control.

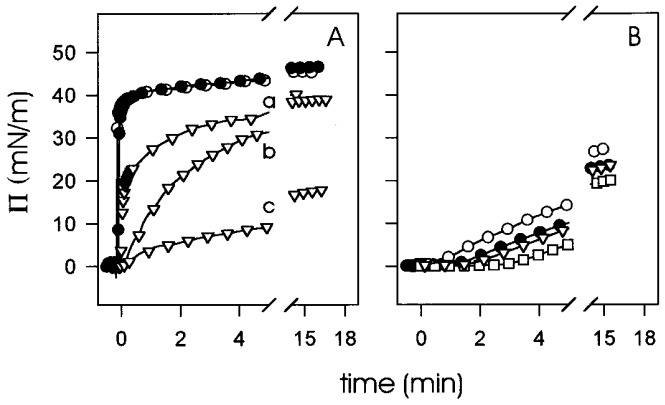

Figure 6. (*A*) Effect of CRP and the effect of CRP + phosphocholine on the adsorption of surfactant from control lungs. *Closed circles*: Surfactant from control lung (45 μg phospholipid/ml). *Triangles*: Surfactant (45 μg phospholipid/ml) + human CRP at a CRP/phospholipid weight ratio of: (*a*) 0.01:1 (0.45 μg CRP/ml), (*b*) 0.05:1 (2.25 μg CRP/ml), and (*c*) 0.3:1 (13.5 μg CRP/ml). *Open circles*: Surfactant (45 μg phospholipid/ml) + human CRP (2.25 μg CRP/ml) + phosphocholine (3 mM). A representative experiment of three experiments from three different control surfactant preparations is shown. (*B*) Effect of phosphocholine and the effect of canine SP-A on the adsorption of surfactant from transplanted lungs. *Closed circles*: Surfactant from transplanted lung (45 μg phospholipid L/ml). *Open circles*: Surfactant (45 μg phospholipid/ml) + phosphocholine (30 mM). *Triangles*: Surfactant (45 μg phospholipid/ml) + canine SP-A (2.5 μg/ml). *Squares*: Surfactant (45 μg phospholipid/ml) + canine SP-A (2.5 μg/ml) + phosphocholine (30 mM). Results presented are from a representative experiment of three experiments from three different surfactant preparations from transplanted lungs.

This is usually termed ischemia-reperfusion injury (1). It is believed that microvascular injury associated to ischemia-reperfusion is due to massive pulmonary polymorphonuclear leukocyte (PMN) adhesion to endothelial cells. Adhesion of PMNs is mediated by CD18 leukocyte integrin during reperfusion and is further increased by the release of pro-inflammatory cytokines by alveolar macrophages (such as tumor necrosis factor-α or interleukin-1β) (26). Activation of adherent neutrophils worsens microvascular injury by causing the release of active oxygen species, proteolytic enzymes, and additional cytokines (27). Once the cascade of events resulting in reperfusion injury is initiated, secondary effects such as alteration of the alveolar surfactant system (5, 6) may complicate the clinical picture. The present study analyzes the composition and activity of the surfactant system after lung transplantation and investigates whether the acute-phase protein CRP increases in surfactant from transplanted lungs.

The first evidence of lung injury in transplanted lungs after long-term preservation was a decrease in arterial P_{O_2} after 4.5 h of reperfusion and increased levels of proteins recovered from BAL, in agreement with other investigators (5, 6). In this study, we partly purified large surfactant aggregates (heavy subtype surfactant) from blood components that leaked into the alveoli. Large surfactant aggregates from lung transplants showed a phospholipid content and composition similar to that of the control group. These results differ from those of Veldhuizen and coworkers (5). They found more sphingomyelin and less phosphatidylglycerol in surfactant from canine transplanted lung. The discrepancies between the current results and those of Veldhuizen and coworkers (5) could be due to the different experimental systems used. We partly purified large surfactant aggregates from serum components. It might be possible that the increase of the serum phospholipid sphingomyelin and the decrease of the surfactant phospholipid phosphatidylglycerol reported by these investigators was a consequence of the edema filling the damaged alveoli.

Despite the purification procedure of large surfactant aggregates, we found an increased protein/phospholipid ratio in transplanted lung with respect to that of control lung. We show here that CRP was one of the nonsurfactant proteins that increased and remained associated with large surfactant aggregates from transplanted lungs preserved with either EC or UW solution. The increase in CRP levels occurred after reperfusion and not during the 22-h ischemic period. CRP severely affects surfactant activity *in vitro* as well as other plasma proteins such as albumin, hemoglobin, and or fibrinogen (12, 16). The presence of elevated levels of CRP in large surfactant aggregates from transplanted lungs could partly explain the decreased ability of these surfactants to adsorb very rapidly from an aqueous subphase into the air–water interface. Increased levels of CRP were also found in BAL from patients with sepsis-induced ARDS and decreased surfactant adsorption was reported (28).

The precise *in vivo* function of CRP remains unclear. Several lines of evidence indicate that CRP might participate in pulmonary host defense (29, 30). Interestingly, a recent study (31) indicated that CRP might inhibit lung inflammation by down-regulating alveolar macrophage production of interleukin-1β in response to endotoxin. CRP seems to suppress inflammation in the alveolar space, but excess CRP might be harmful for the lung function because part of CRP present in the alveolar space would interact with large surfactant aggregates. We show here that CRP decreased the adsorption activity of control surfactant at a low CRP-to-surfactant phospholipid weight ratio (0.01:1). The effect of CRP on surfactant adsorption was effectively reversed by addition of phosphocholine, indicating that CRP binds to phosphocholine headgroups of surfactant lipids as previously suggested (16, 17). Addition of excess phosphocholine to surfactant from lung transplants had no effect on its reduced adsorption activity. The decreased content of SP-A in surfactant from transplanted lungs as well as the contamination with other plasma proteins could also contribute to the diminished adsorption rate of those surfactants. Decreased levels of SP-A were also reported in large surfactant aggregates (5) and in BAL (6) obtained from lung transplants and in ARDS (3, 4). We found that the decrease of SP-A content occurred after reperfusion because the content of SP-A did not decrease in donor lungs after long-term storage with either EC or UW solution. Decreased levels of SP-A and SP-B are likely due to degradation by neutrophil proteases (10) or by increased leakage into the bloodstream (32). SP-B is essential for the surface activity of surfactant. SP-A accelerates the adsorption process, modulates surfactant homeostasis, and participates in host defense. In addition, SP-A performs an essential function when surfactant activity is compromised by the presence of inhibitory agents such as serum proteins. *In vitro* (12) and *in vivo* (33) studies demonstrated that SP-A counteracted serum protein inhibition of surfactant function. The present study revealed that addition of SP-A to surfactant from transplanted lungs did not reverse inhibition of surfactant adsorption. It is likely that SP-A can prevent but not reverse serum protein inhibition. We speculate that instillation of surfactant containing SP-A in the donor just before anastomosis and reperfusion could prevent some of the surfactant changes that occur as a

consequence of ischemia-reperfusion injury and, therefore, improve transplant function.

In conclusion, this study shows that, irrespective of the preservation fluid: (*1*) adsorption facilities of surfactant from transplanted lungs are impaired as well as graft function and (*2*) CRP levels increase in surfactant upon reperfusion whereas the relative content of SP-A significantly decreases. The presence of elevated levels of CRP in BAL could be a very sensitive marker of lung injury. Because most of the changes in the surfactant system seems to occur as a consequence of ischemia-reperfusion injury, we suggest that instillation of exogenous surfactant just before reperfusion might (*1*) protect endogenous SP-A from degradation by increasing the percentage of SP-A bound to lipids, which is less susceptible to proteolytic degradation (34) and (*2*) diminish the CRP/surfactant phospholipid ratio, which would decrease the ability of CRP to inhibit surfactant absorption. Alternatively, new strategies of surfactant treatment could include the presence of SP-A or the water-soluble CRP ligand phosphocholine, which diminishes the inhibition of surfactant adsorption by CRP.

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