Glutathione Decreases the Pulmonary Reimplantation Response in Canine Lung Autotransplants*


The pulmonary reimplantation response (PRR) is a form of membrane permeability pulmonary edema occurring in lung transplants. The severity of the PRR reflects the quality and duration of lung graft preservation. Free radicals formed during ischemia with reperfusion in the autotransplanted dog lung may play a role in producing PRR. We hypothesized that the addition of reduced glutathione (GSH) to the preservative solution could decrease PRR if hydroperoxides are being formed. Six dogs underwent left lung autotransplantation after the lung was flushed with Euro-Collins solution (EC). These dogs demonstrated radiographic and histopathologic evidence of bilateral pulmonary edema, greatest in the transplanted left lung. They also had increases in lung wet to dry weight (W/D) ratios in both lungs (left, 12.0 ± 0.9; right, 10.1 ± 0.8) as compared with a group of five unmanipulated control animals (left, 6.0 ± 0.5; right, 7.0 ± 0.4). Malondialdehyde (MDA) concentrations were significantly increased in the transplanted left lungs (14 ± 4) from this group as compared with the controls (5 ± 7). Five additional dogs underwent left lung autotransplantation with GSH added to the EC cryopreservation fluid. These animals did not develop histologic or radiographic evidence of pulmonary edema, and W/D ratios as well as MDA concentrations were not different from those in controls. To evaluate the effect of ischemia alone on changes in lung GSH concentrations, ten additional dogs underwent left pneumonectomy. Left lungs were cryopreserved in EC+GSH. In five of the animals, the right lung was removed and preserved in EC alone. In the other five animals, the right lung remained in vivo for 3 h and was then removed. Lung GSH concentrations were doubled after 3 h of ischemia when incubated in EC+GSH compared to in vivo controls and to EC-treated lungs. These data suggest that GSH added to the preservation fluid prevents PRR following transplantation and that lung GSH concentrations actually increase during preservation prior to reimplantation and reperfusion if the lung graft is exposed to GSH in the preservation fluid.

(Chest 1991; 100:1694-1702)

Recent improvements in transplantation immunology, surgical technique, and graft preservation have made lung transplantation and lung-heart transplantation feasible in selected patients. In the early postoperative period, reimplanted lungs develop reversible alveolar edema, which has been referred to as the "pulmonary reimplantation response" (PRR). This response has been poorly characterized and theoretically attributed to disruption of nerves, lymphatic vessels, and bronchial vessels or ischemic injury. The PRR may represent a form of ischemia with reperfusion lung injury. Bishop and colleagues have shown that unilateral lung ischemia with reperfusion in a dog model produces bilateral lung injury, which is greatest in the ischemic lung. One possible mechanism of ischemia-induced injury includes activation of oxygen-derived free radicals that are formed during reperfusion with oxygen-rich blood.

Currently, the PRR is kept to a minimum in human subjects by limiting lung graft preservation time to less than 3 h and by limiting fluid intake in the recipient during the early perioperative period. This 3-h preservation limit, however, restricts organ procurement to small geographic areas easily accessible to the lung transplantation centers. Although infection and graft rejection are now the main complications occurring in clinical lung transplantation programs, the PRR remains an important postoperative phenomenon and may contribute to early morbidity. The severity of the PRR appears to reflect the duration and quality of lung graft preservation. Manipulations designed to attenuate the PRR could improve lung graft function as well as increase the time and distance over which organ procurement can occur.

*From the Departments of Medicine (Lung Metabolic Unit) and Surgery (Cardiothoracic Surgery), The University of Texas Health Science Center at San Antonio; the Audie L. Murphy Veterans Administration Hospital; and Wilford Hall Medical Center, San Antonio, Texas. Supported in part by Institutional Review Grant No. 87227-B and an American Lung Association grant.

Reprint requests: Dr. Bryan, Division of Pulmonary Diseases, 7400 Merton Minter Blvd., San Antonio 78284
Siegelman et al have shown that the PRR consists of alveolar edema, which is evident on histologic examination, and alveolar infiltrates, which may be seen on chest roentgenograms. In mongrel dogs, these changes remain for three days after autotransplantation and regress over one to three weeks. Functionally, the PRR impairs ventilation-perfusion relationships in the transplanted lung.\(^9\)

Since PRR develops early in reimplanted lungs, it may represent a form of reperfusion lung injury. The present study was undertaken to characterize the role of exogenous reduced glutathione (GSH) administration in protecting the autotransplanted dog lung against any membrane permeability edema mediated by peroxide or hydroperoxide production. Glutathione protects lungs from oxidant stress.\(^{10}\) Knowledge of GSH-mediated protection occurring in dog lung autografts may give rise to new methods of lung graft preservation, which could improve survival of lung allografts and increase the geographic distance over which lung procurement is possible. We hypothesized that supplying GSH to the transplanted lung could decrease lung injury produced by the PRR. To test this hypothesis, we performed experiments using autotransplantation of single lungs in a dog model.

**METHODS**

**Animals**

Thirty conditioned male mongrel dogs were studied. Two dogs were eliminated due to the discovery of Dirofilaria at the time of surgery. The remaining 28 animals were considered free of heartworms on the basis of the DiFi Test (5 percent false negative, Evsco Pharmaceuticals, Buena, Mich) and postoperative visual inspection. Selection criteria included age under three years, to minimize aging influences on biochemical changes, and weight between 18 and 20 kg, to facilitate surgical ease of lung removal and reimplantation.

Experimental animals were cared for by laboratory animal resources personnel at the University of Texas Health Science Center at San Antonio in compliance with the Animal Welfare Act (PL89-544, as amended), the National Institutes of Health Guide for Grants and Contracts, and the Guide for the Care and Use of Laboratory Animals.

All animal experiments were conducted using barbiturate anesthesia. A right brachial intravenous line was placed, and the patency was maintained with D5 lactated Ringer's solution at a keep-open rate. All final organ harvesting was conducted under level III pentobarbital anesthesia in compliance with the Panel on Euthanasia of the American Veterinary Medical Association.

Experimental animals were ventilated with a Byrd pressure-cycled ventilator at rates of 12 to 16 breaths per minute and peak pressure of 15 to 20 cm H\(_2\)O (titrated to an arterial carbon dioxide tension of 30 to 40 mm Hg). Supplemental oxygen was blended into the circuit resulting in final inspired oxygen fractions of 0.21 to 0.60, titrated to an arterial oxygen tension between 60 and 80 mm Hg.

**Standard Surgical Methods**

In each of the experimental animals, the left lung was removed through a left thoracotomy incision with use of a standard surgical protocol. The lung was then flushed with preservation solution (Euro-Collins [EC] or EC plus glutathione [EC + GSH]) via the pulmonary artery and immersed in a deflated state in cold preservation solution (4°C) for a period of 3 h to simulate ischemic conditions in human lung allografts. The lung was then reimplanted into its original position with reanastomosis of the left main bronchus, pulmonary artery, and vein. A roentgenogram of the chest was obtained after 45 min of reperfusion in experimental animals. After 1 h of reperfusion, the left hilum was cross-clamped, and the reimplanted left lung was rapidly harvested, followed by the right. Control animals were anesthetized with pentobarbital and ventilated during lung harvesting in a fashion identical to that used in the transplant animals. The left lung was first removed en bloc, followed by the right lung. Both lungs were then subjected to standard procedures for tissue preparation, enzyme analysis, wet and dry lung weight determinations, and histopathologic study.

**Methods for Specific Experiments**

Twenty male mongrel dogs were divided into four groups (Table 1). Lung autotransplantation was carried out concurrently in groups 1 and 3 to minimize the effects of training and improvements in surgical technique on the experimental results. The autotransplanted animals were alternated between EC (group 1) and EC + GSH (group 3) cryopreservation to eliminate selection bias. Harvesting of lungs from control animals (group 2) occurred simultaneously with harvesting of lungs from transplanted animals in the experimental surgical facility. The surgeons were blinded to the type of preservation solution. In the autotransplanted animals, markers of free radical activity and markers of lung injury, including wet to dry lung weight (W/D) ratios, radiographic changes, and histopathologic changes, were measured in the transplanted and the nontransplanted lungs. The investigators and technicians responsible for laboratory measurements were blinded to the previous manipulations of the samples.

The first two transplanted animals were eliminated from the study due to changes in surgical technique. Two dogs were removed from the study due to the intraoperative discovery of heartworms, even though initial screening tests for Dirofilaria were negative. Animals studied subsequently were retested for Dirofilaria (DiFi Test) the day prior to surgery. Data on the remaining 26 animals are reported.

Reduced glutathione (product No. C4251) was obtained from laboratory suppliers. Glutathione was reconstituted in saline to a concentration of 250 mg/ml.

**Table 1—Experimental Design**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Group 1 (n = 6)</th>
<th>Group 2 (n = 5)</th>
<th>Group 3 (n = 5)</th>
<th>Group 4 (n = 10)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Right</td>
<td>Left</td>
<td>Right</td>
<td>Left</td>
</tr>
<tr>
<td>Ischemia duration, h</td>
<td>N/A</td>
<td>3</td>
<td>N/A</td>
<td>3</td>
</tr>
<tr>
<td>Reperfusion duration, h</td>
<td>N/A</td>
<td>1</td>
<td>N/A</td>
<td>1</td>
</tr>
<tr>
<td>Preservation solution</td>
<td>N/A</td>
<td>EC</td>
<td>N/A</td>
<td>EC</td>
</tr>
</tbody>
</table>

* N/A = not applicable.
Sigma (St Louis). Eu o-Collins solution was reconstituted from electrolyte solution for kidney preservation (Baxter Healthcare Dialysis Division, Deerfield, Ill by adding 65 ml of 50 percent dextrose. For GSH-supplemented cryopreservation, GSH was dissolved in sterile water and added to 1 L of EC solution to reach a final concentration of 1 mg/ml. This concentration of GSH has been added to cryopreservation solutions by others to decrease reperfusion injury to liver and kidney transplants. The EC + GSH solution was used to flush the lung graft and for cryopreservation in groups 3 and 4.

Group 1 animals (n = 6) were subjected to standard experimental procedures. These dogs underwent left lung autotransplantation, as already described. The left lung was flushed with and submerged in EC solution at 4°C during the 3-h ischemic cryopreservation period. One hour after reimplantation, the animals underwent left pneumonectomy followed by right pneumonectomy while still under anesthesia.

Group 2 animals (n = 5) served as unmanipulated controls. Lungs and blood were harvested with the animals under anesthesia, as already described without cryopreservation or transplantation.

Group 4 animals (n = 5) underwent left lung autotransplantation with 3 h of ischemic cryopreservation and 1 h of reperfusion as in group 1 except that GSH was added to the EC flushing and cryopreservation solution.

Group 4 animals (n = 10) were studied to evaluate GSH uptake in the ischemic lung without reperfusion. These animals underwent left pneumonectomy under conditions identical to those in transplanted animals. The left lung from each animal was flushed and preserved in EC supplemented with GSH (1 mg/ml) for 3 h. Five of the animals underwent immediate right pneumonectomy, and the lung was preserved with EC alone for 3 h to determine ex vivo lung GSH concentrations prior to reimplantation. The remaining five animals were maintained under barbiturate anesthesia for 3 h. The right lung was then removed and flushed free of blood with normal saline solution and subjected to GSH measurements to determine whether in ex vivo lung GSH concentrations changed prior to reimplantation. All group 4 lungs were therefore flushed with normal saline solution after 3 h and subjected to analysis for GSH measurement, as already described.

**Monitoring**

The arterial to alveolar oxygen tension ratio (Pa/oA2) was measured at baseline, at two points after initiation of ischemia, and at two points after initiation of reperfusion, and the values for dogs treated with EC alone and those treated with EC + GSH were compared. The Pa/oA2 is a stable index of gas exchange function which compensates for changes in inspired oxygen and changes in ventilation. The value was obtained by dividing the arterial oxygen tension by the estimated alveolar oxygen tension (from the alveolar air equation).

During the ischemic cryopreservation period, all transplanted animals were monitored under level III barbiturate anesthesia with continuous electrocardiographic, arterial pressure, and central venous pressure or pulmonary arterial wedge pressure measurements. The central venous pressure never exceeded 5 mm Hg, and the pulmonary arterial wedge pressure never exceeded 12 mm Hg in any of the monitored animals. All animals received less than 500 ml of Ringer's lactate during the 6-h procedure to avoid hypervolemia. Systemic systolic arterial pressures were maintained at 120 to 180 mm Hg.

**Standard Tissue Preparation**

During perfusion, serial blood samples were obtained for the measurement of thiobarbituric acid (TBA) reactants. At the time of sacrifice, anesthesia was maintained, animals were subjected to thoracotomy, and a 20-ml aliquot of blood was collected. Bilateral pneumonectomies were completed, and the lungs were weighed.

The lungs were then separated into their constituent lobes and trimmed of extraparenchymal tracheobronchial tissue. The lower lobe from each lung (normal lung and graft) was perfused free of blood with use of cooled normal saline solution by cannulation of the lobar pulmonary arteries. A lower lobe segment was immediately resected, freeze-clamped, weighed, and assayed for glutathione. Portions of the perfused lobe were homogenized in 0.25 molar sucrose. The 90 percent lung homogenates and blood were then processed in parallel fashion for biochemical studies.

The upper, middle, and/or accessory lobes were not perfused free of blood in order to measure accurate wet and dry lung weights and histopathologic changes.

**Standard Biochemical Methods**

Catalase assays were performed on blood and fresh lung homogenates with use of the spectrophotometric method of Beers and Sizer. Portions of homogenates and blood were sonicated to break up mitochondria and were centrifuged at 2,500g. The soluble fraction was then collected for superoxide dismutase (SOD) assays using the method of McCord and Fridovich. Portions of fresh homogenate and blood were centrifuged at 105,000g, and the soluble fractions were collected for the other assays. Measurement of total glutathione peroxidase (GSH-Px) activity was performed by means of the coupled assay of Paglia and Valentine as modified by Lawrence and Burk using cumene hydroperoxide as substrate. All enzyme levels were expressed per milligram of DNA according to the method of Abraham et al.

**Malondialdehyde Measurements**

Malondialdehyde (MDA) was measured as TBA reactants in tissue quickly frozen in liquid nitrogen according to the method of Uchiyama and Miura. Portions of the frozen lung were homogenized in hydrochloric acid and phosphoric acid. The samples were then heated for 45 min and extracted with N-butanol. The butanol phase was separated by centrifugation, and absorbance was measured at 535 and 520 nm. The difference was used as the TBA value and reported in nanomoles per milligram of DNA.

**Glutathione Measurements**

Immediately on removal of the lungs, a lobe was removed and perfused, and segments were freeze-clamped at liquid nitrogen temperature. The frozen tissues were homogenized in 10 percent trichloroacetic acid to precipitate protein.

The total glutathione (T-GSH) level (GSH and glutathione disulfide [GSSG]) was measured by the recycling method of Tietze, utilizing 5,5'-dithiobis-2-nitrobenzoic acid. Blood concentrations of T-GSH were determined by the method of Adams et al.

To correct for blood contamination of tissue samples, which could result in factitious elevation of glutathione, SOD, catalase, or GSH-Px values, these parameters were measured in whole blood and expressed in micromoles or units of activity per milligram of hemoglobin. Hemoglobin concentrations were then determined in the tissue homogenates, and the glutathione or enzyme activity contributed by extravasated blood was subtracted from the total tissue amount. Hemoglobin concentrations were determined by the method of Harboe.

**Standard Morphologic Methods**

Roentgenograms of the chest were obtained in the postoperative period after 45 min of reperfusion, using frontal and lateral projections of the animal in the prone position. Radiographs were reviewed at the end of the study by a reader who was blinded to the method of preservation. Each lung (right and left) from the transplanted animals was assigned a radiographic score for the severity of edema based on the scale developed by Pistolesi and colleagues. Wet and dry weights of the transplanted and nontransplanted lungs of each dog were measured. The right and left lungs...
were weighed separately. A portion of each lung was resected, weighed, desiccated, and reweighed. A lobe of each lung for histologic study was cannulated via the lobar bronchus, inflated at 20 cm H₂O pressure, and fixed by submersion in Carnoy's solution. After fixation, the lobes were sliced transversely at 1-cm intervals from base to apex. Slices were taken at four levels from each lobe. Tissue was processed for paraffin hematoxylin-eosin sections for light microscopy. The pathologist reading the tissue sections was blinded to the treatment groups.

**Standard Statistical Methods**

Enzyme activities, GSH and MDA concentrations, and W/D weight scores for the transplanted and nontransplanted lungs were compared in each animal using Student's paired t test. The Duncan's multiple-range test was used to compare differences in measured variables among EC-preserved animals, GSH-treated animals, and controls. Repeated-measures analysis of variance was used to compare PaO₂ ratios between EC-preserved animals and GSH-treated animals.

**RESULTS**

We first determined the effects of lung transplantation under the conditions stated in our Methods section on producing the PRR in group 1 dogs. Radiographic signs of bilateral pulmonary edema were present. Dorsoventral chest x-ray films from transplanted animals demonstrated diffuse alveolar consolidation with air bronchograms in the transplanted lungs compared with those from nontransplanted control animals. The nontransplanted right lungs demonstrated less severe edema, manifested in the transplanted dogs as effacement of right vascular shadows and silhouetting of the right middle lobe fissures. Therefore, pulmonary edema occurred in both the transplanted left lung and the nontransplanted right lung. From these data, we concluded that our model did produce the PRR.

Group 2 dogs (controls) underwent bilateral pneumonectomies under barbiturate anesthesia, and group 3 dogs (EC + GSH) were studied after left lung autotransplantation, as already described.

Histopathologic findings in the transplanted left lungs preserved with EC (group 1) were compared with the histopathologic findings in the unmanipulated left control lungs (group 2) and the GSH-treated lungs (group 3). The EC-preserved lungs were noted to have diffuse interstitial thickening (Fig 1, left) as compared with control (Fig 1, center) and GSH-treated (Fig 1, right) lungs. This thickening was characterized by infiltrates of acute and chronic inflammatory cells and extravasated erythrocytes, as well as stromal edema, which was not present in the control lung or in the lung treated with GSH. Therefore, reperfusion resulted in marked edema and inflammatory infiltrates in the transplanted lungs, changes which were not found in lungs cryopreserved with GSH. Histopathologic study of nontransplanted right lungs of EC animals (group 1) demonstrated inflammatory infiltrates and edema, but these findings were not as marked as those in the transplanted left lung. Radiographic scores were also compared between EC-preserved animals (group 1) and EC + GSH-preserved animals (group 2). Two dorsoventral radiographs from group 1 and one dorsoventral radiograph from group 2 were not scored due to poor technical quality. Left

![Figure 1. Representative inflated lung sections. Transplanted left lungs of EC-preserved animals (left) had diffuse interstitial thickening compared with control left lungs (center) and transplanted lungs treated with EC + GSH (right). The interstitial thickening in EC-preserved animals was characterized by infiltrates of acute and chronic inflammatory cells and extravasated erythrocytes, as well as stromal edema, which was not present in control or EC + GSH-treated lungs (hematoxylin-eosin, original magnification ×10).](http://journal.publications.chestnet.org/pdfaccess.ashx?url=/data/journals/chest/21637/)
and right radiographic edema scores from EC animals were 18 and 9, respectively, compared to 6 and 5 in the comparable lungs from EC + GSH animals (p<0.05). In general, the higher the score, the greater the amount of radiographic pulmonary edema. Radiographic scores greater than 8 have been associated with significant edema and hypoxemia in clinical studies of adult respiratory distress syndrome.21

The W/D ratios in all three groups of animals were also compared (Fig 2). The W/D ratios in group 1 animals were significantly greater in the transplanted left lung than in the nontransplanted right lung (p<.05). The W/D ratios in both lungs from the group 1 animals were greater than those in controls (p<.001); therefore, single-lung transplantation produced bilateral pulmonary edema. Dogs treated with GSH (group 3) demonstrated W/D ratios in the transplanted left lung and in the nontransplanted right lung that were not different from those in controls. Therefore, adding GSH to the ex vivo flushing/cryopreservation solution of the transplanted lung prevented pulmonary edema formation in both lungs.

The MDA concentration was measured in tissue from both lungs to assess lipid peroxidation in groups 1–3 (Fig 3). Levels of TBA reactants were higher in both lungs from transplanted animals treated with EC cryopreservation alone (group 1) than in the control group (group 2) or in the GSH-cryopreserved group (group 3). Levels of TBA reactants were statistically greater in the EC-preserved transplanted left lung than in the right lung from group 1 animals at p<.05. Therefore, lipid peroxidation occurred in both lungs from group 1 animals treated with EC alone, and lipid peroxidation was higher in the EC-cryopreserved transplanted lung than in the nontransplanted right lung. The addition of GSH to the ex vivo flushing/cryopreservation solution prevented any increase in TBA reactants. The MDA concentration was also measured in plasma during reperfusion. All plasma MDA concentrations were below the level of sensitivity for the assay.

The Pa/AO2 ratios of the two groups of transplanted
animals (groups 1 and 3) were compared (Fig 4). There were no significant differences in gas exchange at baseline and during the 3-h period of ischemic cryopreservation. After reimplantation, Pa/O_2 ratios were significantly greater in the animals treated with GSH than in the animals treated with EC alone (p < 0.03). The higher Pa/O_2 ratios in group 3 animals suggest that GSH diminishes the deterioration in gas exchange that occurs during reperfusion.

Lung GSH-Px, catalase, and total SOD activities were measured in blood and both lungs from the first three groups of animals to characterize any changes (Table 2). There were no statistically significant differences in lung GSH-Px, catalase, or total SOD activities among the three groups of animals.

Total glutathione levels were measured in both lungs of animals from groups 1, 2, and 3 and expressed in nanomoles per gram of lung weight (Fig 5). The lung T-GSH levels were higher in both of the autotransplanted groups than in controls (p < 0.01). Within each of the transplanted groups, the native right lung was higher in T-GSH than the transplanted left lung. There were no differences in lung T-GSH levels between dogs preserved with EC alone and those preserved with EC + GSH after 3 h of ischemia and 1 h of reperfusion.

Because of the finding that the lungs reimplanted in the experimental animals showed an increase in T-GSH concentration after 1 h of reperfusion, we performed a final experiment measuring changes in lung glutathione after 3 h of ischemia without reperfusion to investigate whether the T-GSH increase occurred prior to reimplantation. The left lung from each animal in group 4 was flushed with and submerged in EC + GSH. The right lung from half the animals was flushed with and submerged in EC alone (ex vivo). All left lungs and ex vivo right lungs were subjected to 3 h of ischemia. The right lung from the other half of group 4 animals remained in vivo after 3 h prior to removal. At the end of the ischemic period, all lungs were flushed with normal saline solution via the pulmonary artery. The T-GSH concentration was then measured in all lungs and expressed in nanomoles per gram of lung weight. The T-GSH concentrations were significantly elevated in ischemic lungs preserved with EC + GSH as compared with those submerged in EC without GSH and those that remained in vivo (p < 0.01) (Fig 6). Lungs preserved in EC without GSH had T-GSH concentrations similar to those of in vivo controls.
These data reveal that the ischemic lung is capable of increasing tissue T-GSH concentrations if GSH is added to the EC solution. Protection afforded by exogenous GSH in dog lung autotransplants may be related to increases in lung T-GSH prior to reperfusion, since animals treated with either EC + GSH or EC alone both demonstrate high lung GSH levels after reperfusion. Only the EC + GSH-treated animals had increased lung T-GSH concentrations before reperfusion, and these were the only animals protected from the PRR.

**DISCUSSION**

The PRR is a reproducible model of membrane permeability edema first described in a dog lung autotransplant model by Siegelman and colleagues. It appears to occur to some degree in all lung transplants in which warm ischemia persists for over 30 min or cold ischemia persists for over 2 h. The PRR does occur in humans receiving lung transplants. It is best examined experimentally in lung autografts, since allograft transplantation can result in transplant rejection phenomena that are clinically similar to the PRR. The severity of the edema as measured by the amount of edema and the time to resolution appears to vary with the ischemic time and the duration of reperfusion. In human lung transplants, the PRR significantly limits the extracorporeal ischemic preservation time to 3 h. Interventions that attenuate the PRR should allow better immediate function of the lung and increase the time of extracorporeal lung graft preservation.

Glutathione is an intracellular tripeptide that is oxidized in the presence of the enzyme glutathione peroxidase to reduce hydrogen peroxide or lipid hydroperoxides to less toxic compounds. The oxidation reaction results in the formation of GSSG, which can then be reduced by glutathione reductase to reform GSH at the expense of intracellular NADPH. It is preferentially more available to oxygen radicals than enzyme thiol groups. High levels of GSH may serve to protect intracellular enzymes from inactivation and detoxify hydrogen peroxide and lipid peroxides. Glutathione may also reactivate enzymes that have previously been inactivated during oxidant stress.

In these experiments, we found that the addition of GSH to EC cryopreservation solution resulted in decreases in the PRR following autotransplantation. The EC + GSH-treated animals exhibited less pulmonary edema than the EC-treated group following transplantation, as evidenced by decreased edema on chest x-ray examination and decreased W/D ratios. The EC + GSH-treated animals also had fewer TBA reactants formed in the lungs following transplantation as compared with the EC without GSH group. These data suggest that the addition of GSH to the cryopreservation solution protects the lung from injury following reimplantation and decreases the PRR.

The decrease in posttransplantation lung injury afforded by exogenous GSH administration was also associated with an improvement in gas exchange function. During reperfusion after reimplantation, the Pa/A0₂ dropped by 55 percent from baseline in EC-preserved animals, whereas animals treated with EC + GSH experienced a drop in the Pa/A0₂ of only 20 percent.

Our studies revealed that both the right and left lungs were affected by the PRR following reimplantation of the left lung autograft in the EC group. This finding supports previous work by Bishop et al., demonstrating that unilateral lung ischemia with reperfusion produces bilateral lung injury. Increased levels of lung water and TBA reactants also occurred in the right lungs of the EC group despite the fact that they were not autotransplanted. These data suggest that a circulating mediator may have been produced in the transplanted left lung which affected the nontransplanted right lung during reperfusion. Markers of lipid peroxidation may have been filtered out in the nontransplanted lungs due to crossed circulation. However, the absence of a significant MDA concentration in plasma suggests that injury truly occurred in the nontransplanted lung. The addition of GSH to the EC used to preserve the transplanted lung abolished these effects in both lungs.

The current standard preservation technique for human lung transplantation is to flush and submerge the deflated graft with EC at 4°C. Antioxidants added to the flushing and cryopreservation solution of heart and kidney transplants have been shown to
result in increased protection of normal organ morphology and preservation of normal function.\textsuperscript{35,36} Antioxidants may be administered during the donor operation, added to the flushing solution, or infused into the recipient organ prior to restoration of organ blood flow to improve graft preservation.

We chose to add GSH to the cryopreservation solution in order to compartmentalize this antioxidant in the area of greatest oxidant stress, i.e., the ischemic lung graft. Fuller and Green\textsuperscript{37} reported decreased lipid peroxidation in rabbit kidney allografts when deferoxamine was added to the flush solution at a 50-mmol concentration. This compound is thought to decrease free radical production by binding transitional metal catalysts. When deferoxamine, 15 mg/kg, was infused into the donor organ just prior to reimplantation, lipid peroxidation was decreased after 1 h of reperfusion but rose again after 24 h. The prevention of free radical formation may be extremely important in abolishing the PRR. Recently, Kennedy et al.\textsuperscript{37} confirmed the production of superoxide anion in an isolated rabbit lung model of ischemia with reperfusion injury. They also demonstrated a reduction in injury by various antioxidants, including the thiols dimethylthiourea and N-acetylcysteine. Jenkinson et al.\textsuperscript{38} have reported oxidation of GSH in rat lung subjected to hypoxia followed by reoxygenation, suggesting that glutathione may undergo oxidation-reduction during lung ischemia with reperfusion.

The tissue T-GSH levels were significantly increased after reperfusion in both groups of transplanted animals as compared to controls. The T-GSH level was actually highest in the nontransplanted lung in both the EC and the EC + GSH groups, suggesting that mechanisms turned on to increase lung GSH during reperfusion were not as active in the transplanted lung. Total glutathione was induced in both lungs of the group 1 animals after reperfusion as compared to controls. Despite this increase in GSH, these animals developed the PRR as assessed by chest radiographic evidence of pulmonary edema and increases in lung water. Lung GSH in group 3 animals was also increased to levels similar to those in group 1 animals, but these dogs did not develop the PRR following reimplantation. These data suggest that lung GSH needs to be induced prior to reimplantation in order to prevent the PRR. Previous studies have shown that pulmonary epithelial cells and other cells exposed to oxidant stress will develop increased cellular glutathione concentrations within minutes to hours after treatment with a glutathione-containing solution.\textsuperscript{39-41}

The T-GSH levels increased in EC + GSH-treated lungs by almost 75 percent after 3 h of ischemia without reperfusion. This increase in lung glutathione may have been due to increased cellular uptake, increased retention in extracellular tissue fluid, or decreased washout from the intravascular space by the saline flush at the end of the ischemic period. Lung GSH-Px, catalase, and SOD activities were not significantly different among any of the experimental or control groups of animals. Therefore, the protection from posttransplant lung injury afforded by exogenous GSH was not mediated by changes in these lung antioxidant enzyme activities.

The use of GSH to decrease the PRR following transplantation may be important in prolonging the time of ex vivo lung graft preservation. Hardy and his associates\textsuperscript{42,43} have reported that a 2-h interval of total ischemia at 4°C is the limit for preservation without damage, whereas a 4-h interval is the limit for preservation with reversible damage in dog lungs. The addition of GSH to EC preservation solution could increase the distance over which donor lungs can be procured if GSH-induced protection increases the time limit for graft preservation. Increasing the time of graft preservation could ultimately increase the number of donor lungs available to a particular transplant center.

REFERENCES

7. Meerson FZ, Kagan VE, Kozlov YE, Belki YY. The role of lipid peroxidation in pathogenesis of ischemic damage and the antioxidant protection of the heart. Basic Res Cardiol 1982; 77:465-85
15. Lawrence RA, Burk RF. Glutathione peroxidase activity in selenium-deficient rat liver. Biochem Biophys Res Commun
Glutathione Decreases the Pulmonary Reimplantation Response (Bryan et al)

19 Tietze F. Enzymic method for quantitative determination of nanogram amounts of total and oxidized glutathione: applications to mammalian blood and other tissues. Anal Biochem 1969; 27:502-22
24 Einot I, Gabriel KR. A study of the powers of several methods of multiple comparisons. J Am Stat Assoc 1975; 70:574-83
41 Olson CE. Glutathione modulates toxic oxygen metabolite injury of canine chief cell monolayers in primary culture. Am J Physiol 1968; 254:G49-G56