CARDIAC AND PULMONARY REPLACEMENT

EFFECTS OF ACUTE REJECTION AND ANTIREJECTION THERAPY ON ARTERIES AND VEINS FROM CANINE SINGLE LUNG ALLOGRAFTS

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Experiments were designed to compare the function of the endothelium and smooth muscle in intralobar pulmonary arteries and veins of transplanted lungs during acute rejection and after treatment of rejection. Single lung allografts were performed in dogs. Dogs were monitored for 5 days to allow good recovery from the operation and resolution of early chest radiographic changes. In group I, immunosuppression (cyclosporine A, azathioprine, and methylprednisone) was withdrawn to allow rejection, which typically occurred after 3 days. In group II, immunosuppression was reinstituted at this time during acute rejection until the chest roentgenograms again cleared (approximately after 6 days). The blood vessels were studied at this time. Rings were cut from intralobar pulmonary arteries and veins of the allotransplanted lungs and suspended for the measurement of isometric force in organ chambers. Contractions of arteries and veins to phenylephrine but not endothelin-1 were significantly reduced during acute rejection. In arteries and veins, endothelium-dependent relaxations to bradykinin but not the calcium ionophore A23187 were reduced with rejection. Relaxations of the smooth muscle to histamine increased with rejection in both blood vessels. Relaxations to nitric oxide were reduced with rejection in veins but not arteries. Treatment of rejection reversed all responses toward those observed in arteries and veins in lungs from dogs not undergoing transplantation. These results suggest that responses of the endothelium and smooth muscle of pulmonary arteries and veins of transplanted lungs are altered similarly during rejection. Further, treatment of rejection restores function of the pulmonary blood vessels of lung allografts toward that observed in unoperated lungs. (J Thorac Cardiovasc Surg 1996; 111:1219-29)

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Lung transplantation is now an established therapy for selected patients with end-stage lung disease.[1-3] Episodes of acute rejection continue to occur in the early postoperative period. Rejection begins as a perivascular phenomenon.[4] Endothelium-dependent relaxations in pulmonary arteries of allotransplanted lungs are decreased during episodes of acute allograft rejection. These changes are distinct from those caused by denervation alone after autotransplantation.[5] Effects of rejection on the function of pulmonary veins are not known. Further, during treatment of rejection lymphocytic infiltrates disappear from the perivascular space. However, it is not known whether endothelium-dependent responses are restored with reversal of rejection. Therefore, the aim of this study was (1) to

characterize endothelium-dependent responses of the pulmonary veins during acute rejection of lung allografts and (2) to determine whether functional changes in pulmonary arteries and veins are reversible with treatment of rejection.

Material and methods

Humane animal care. All animals have received humane care in compliance with the "Principles of Laboratory Animal Care" formulated by the National Society for Medical Research and the "Guide for the Care and Use of Laboratory Animals" prepared by the Institute of Laboratory Animal Resources and published by the National Institutes of Health (NIH Publication No. 86-23, revised 1985).

Operating procedures. Thirteen mongrel dogs of either sex (weight 25 ± 1.8 kg) underwent single left or right lung allotransplants as previously reported.[5] Donors and recipients were matched for size and weight. General anesthesia was induced with sodium methohexital (Brevital, 12 mg/kg intravenously) and was maintained with halothane (at 1% to 2% inspired concentration). Donor lungs were flush-perfused with cold (4° C) Euro-Collins solution (60 ml/kg). Total ischemic time for the transplanted lungs was 75 ± 3 minutes (n = 13).

Immunosuppression and antibiotics. All drugs were given through an indwelling catheter inserted in the right atrium via the external jugular vein. Methylprednisone (125 mg, intravenously) was given at the time of reperfusion of the transplanted lung. Allotransplanted dogs were immunosuppressed with cyclosporine A (4 mg/kg per day plasma concentrations ranged between 200 and 400 mg/L), azathioprine (2 mg/kg per day), and methylprednisone (0.5 mg/kg per day) intravenously. When the roentgenogram of the transplanted lung cleared (usually 5 days after the operation), immunosuppression was discontinued. After an additional 3 days, chest roentgenograms were repeated. When new opacification, indicating acute rejection, of the transplanted lung was apparent, either the dogs were studied (group I: allotransplanted, rejecting) or antirejection immunosuppressive treatment (cyclosporine A, 4 mg/kg per day; azathioprine, 2 mg/kg per day; and methylprednisone, 15 mg/kg per day) was reinstituted until chest roentgenograms again cleared (group II: allotransplanted, treated). This usually took approximately 6 days. Antibiotics (gentamicin, 80 mg/day; clindamycin, 600 mg/day) were continued throughout the study period.

Histology. Transbronchial biopsy specimens were taken from dogs assigned to group II (allotransplanted, treated) 3 days after discontinuation of immunosuppressive treatment for detection of the grade of rejection. When the lungs were removed for organ chamber experiments, (postoperative day 8 for group I and postoperative day 14 for group II), the upper lobes of the lungs were infused with 10% buffered formalin for histologic assessment. Sections stained with hematoxylin and eosin were graded for rejection according to the recommendations of the Society for Heart and Lung Transplantation[4] by an experienced pathologist blinded to the animal's group assignment. Mechanical removal of the endothelium was confirmed by light microscopy of sections cut from rings used in organ chamber experiments.

Organ chamber experiments. The heart-lung block was excised from anesthetized animals (pentobarbital sodium, 30 mg/kg). The intralobar artery and vein from the lower lobe were dissected and placed in modified Krebs-Ringer bicarbonate solution (millimoles per liter): NaCl 118.3, KCl 4.7, $CaCl_2$.5, $MgSO_4$ 1.2, KH_2PO_4 1.2, $NaHCO_3$ 25.0, calcium disodium edetate 0.26, and glucose 11.1 (control solution). Coronary and renal arteries from dogs undergoing transplantation were studied in other experiments. Inasmuch as the goal of organ transplantation is to restore function of a diseased organ, pulmonary arteries and veins from dogs (n = 6) not undergoing transplantation were studied as a reference for responses of blood vessels from healthy animals. All blood vessels were dissected and cut into rings 4 to 5 mm long. In some rings, the endothelium was removed deliberately by gently rubbing the luminal surface with the tip of a pair of forceps. Rings were suspended between a clip and a force transducer (Gould UC-2, Gould Instrument Systems, Inc., Valley View, Ohio) for the measurement of isometric force by two stainless steel wires inserted into the lumen of the ring. The rings were then placed in organ chambers filled with 25 ml of the control solution at 37° C and bubbled with 95% oxygen and 5% carbon dioxide. They were equilibrated at a passive tension of less than 1 gm for 30 minutes. After this time each ring was stretched progressively to the optimal point on its length-tension curve as determined by the tension developed to potassium chloride (20 mmol/L) at each level of stretch. Rings with and without endothelium were studied in parallel. Maximal contraction to a 60

Table I. *Histologic grade of rejection of the allotransplanted lungs**

	Grade of rejection				
	0	*1*	*2*	*3*	*4*
Allotransplanted, rejecting		2	1	1	1
Allotransplanted, treated					
Bioassay during rejection† (postoperative days 8 and 9)		1	3	2	
After treatment of rejection (postoperative days 13 to 15)	1	1	6		

*Values represent number of dogs.

† In two dogs, the biopsy samples were inadequate to grade rejection.

mmol/L concentration of potassium chloride was measured.

After another equilibration period of 30 minutes, cumulative concentration-response curves were obtained to phenylephrine (10^{-9} to 10^{-5} mol/L), angiotensin I (10^{-9} to 10^{-6} mol/L), and endothelin-1 (10^{-10} to 10^{-7} mol/L). To study relaxations, a submaximal concentration of phenylephrine (10^{-6} to 3×10^{-6} mol/L) was used to contract rings of arteries; veins were contracted with prostaglandin $F_{2\alpha}$ (2×10^{-6} mol/L). Canine pulmonary arteries do not contract to prostaglandin $F_{2\alpha}$; therefore, a concentration of phenylephrine was used to produce between 30% and 50% of the maximal contraction. Cumulative concentration-relaxation curves to histamine (10^{-9} to 10^{-4} mol/L) and bradykinin (10^{-10} to 10^{-6} mol/L) were obtained. Relaxations to the calcium ionophore A23187 (10^{-9} to 10^{-6} mol/L) were obtained only in rings with endothelium and relaxation to nitric oxide (3×10^{-9} to 10^{-5} mol/L) in rings without endothelium during contractions to endothelin-1 (10^{-7} mol/L). Before endothelin-1 was added, the rings were incubated with phentolamine (10^{-6} mol/L) and propranolol (5×10^{-6} mol/L).

Drugs used in organ chamber studies were selected for the following reasons: Potassium chloride directly depolarizes the smooth muscle; phenylephrine is an α_1-adrenergic agonist associated with sympathetic innervation; angiotensin I requires the endothelial ectoenzyme angiotensin-converting enzyme[6]; histamine is released from activated mast cells that participate in the inflammatory responses[7]; and bradykinin causes receptor-coupled release of endothelium-derived factors. Endothelin-1 is an endothelium-derived contracting factor that may be released during acute rejection of lung allografts.[8, 9] The calcium ionophore A23187 releases endothelium-derived factors by mechanisms that do not involve receptors. Nitric oxide was tested because it is an endothelium-derived relaxing factor.

Chemical and drugs. The following drugs (all from Sigma Chemical Co., St. Louis, Mo., except where specified) were used: angiotensin I, bradykinin, calcium ionophore A23187, endothelin-1 (Peptide International, Inc., Louisville, Ky.), histamine, phenylephrine, phentolamine (Ciba Pharmaceutical Co., Summit, N.J.), propranolol, and prostaglandin $F_{2\alpha}$. Drugs were prepared daily and kept on ice. A23187 was dissolved in dimethylsulfoxide (final bath concentration: 8.2×10^{-3} mol/L). All other drugs were dissolved in distilled water.

Nitric oxide from a cylinder (Union Carbide, Chicago, Ill.) was used to fill a glass gas bulb fitted with a silicone injection septum. A volume of gas was removed with a glass syringe and injected into another glass gas bulb that had been filled with 100 ml of distilled water (bubbled with helium for approximately 3 hours) to give stock solutions of nitric oxide (4×10^{-5} mol/L; 4×10^{-4} mol/L; and 4×10^{-3} mol/L).

Concentrations of the drugs are reported as the final molar (moles per liter) concentration in the organ chamber.

Calculations and statistical analysis. Results are expressed as means ± standard error of the mean. In all experiments, n equals the number of animals from which rings were taken. Where appropriate, the effective concentrations causing 50% of maximal responses (ED_{50}) were calculated for individual concentration-response curves and the mean of these values was reported as the negative logarithm of the molar concentration. Because rings with and without endothelium of the same blood vessel were studied in parallel, Student's t test for paired observations was used within treatment group. Analysis of variance was used to compare means among groups. When a significant value was obtained, Scheffe's test was used to identify differences among means. Values were considered to be statistically different when p was less than 0.05.

Table II. *Maximal contractions of pulmonary arteries and veins from unoperated and allotransplanted lungs to potassium chloride (60 mmol/L)*

	With endothelium (gm)	*Without endothelium (gm)*
Arteries		
Unoperated (n = 6)	5.0 ± 0.4	5.3 ± 0.4
Allotransplanted, rejecting (n = 5)	4.2 ± 1.0	4.9 ± 0.7
Allotransplanted, treated (n = 8)	4.6 ± 0.6	4.2 ± 0.8
Veins		
Unoperated (n = 6)	3.5 ± 0.4	3.6 ± 0.2
Allotransplanted, Rejecting (n = 5)	2.0 ± 0.5*	1.7 ± 0.2*
Allotransplanted, Treated (n = 8)	3.3 ± 0.7	3.0 ± 0.5

Values shown as means ± standard error of the mean; n = number of animals studied.

*Statistical significance from control and treated groups (analysis of variance, $p < 0.05$).

Results

The histologic grade of rejection is given in Table I. After withdrawal of immunosuppression, chest roentgenograms of all transplanted lungs opacified. Chest roentgenograms cleared when immunosuppression was reinstituted in all animals of group II (allotransplanted, treated). Histologically, after reinstitution of antirejection immunosuppression, residual but lesser rejection was present in animals of group II.

Pulmonary veins. Potassium chloride (60 mmol/L) caused comparable contractions in pulmonary veins with and without endothelium. Maximal contractions to potassium chloride were reduced significantly during rejection in pulmonary veins; the contractions were restored with treatment of rejection (Table II).

Phenylephrine caused concentration-dependent contractions in pulmonary veins from all groups of dogs (Fig. 1). In unoperated veins, contractions to phenylephrine were less in rings with endothelium compared with those without endothelium (Fig. 1). With rejection, contractions of rings with and without endothelium were reduced significantly ($p < 0.05$) compared with those of unoperated veins, and the difference in contractions between rings with and without endothelium was no longer observed. In veins, treatment of rejection increased and restored differences in contraction between rings with and without endothelium (Fig. 1). None of the pulmonary veins from any group of dogs contracted to angiotensin I. In contrast, endothelin-1 caused concentration-dependent contractions in all veins. However, these contractions were not affected significantly by either rejection or treatment of rejection (Fig. 2).

In pulmonary veins contracted with prostaglandin $F_{2\alpha}$, histamine caused slight relaxation (<8%) in lower concentrations and contraction in higher concentrations ($>10^{-5}$) in veins with and without endothelium from unoperated lungs. With rejection, the degree of relaxation was increased significantly ($p < 0.05$) compared with that of unoperated veins. These relaxations were reduced significantly with treatment of rejection ($p < 0.05$; Fig. 3).

Bradykinin caused endothelium-dependent relaxations of pulmonary veins from unoperated and treated dogs. With rejection, relaxations of pulmonary veins with and without endothelium were comparable, and at concentrations greater than 3×10^{-8} mol/L contractions were observed (Fig. 4).

The calcium ionophore A23187 and nitric oxide caused concentration-dependent relaxations in veins with endothelium and without endothelium, respectively. Relaxations to the ionophore were not altered significantly either with rejection or with treatment of rejection (Table III). However, maximal relaxations to nitric oxide were reduced during rejection in venous rings without endothelium (Table III). This effect was reversible with the treatment of rejection.

Pulmonary arteries. Maximal contractions to potassium chloride were not different between arteries with and without endothelium and were not altered during rejection or treatment of rejection (Table II). Contractions to phenylephrine were also comparable in rings with and without endothelium (Fig. 5). With rejection, contractions of both rings with and without endothelium to phenylephrine were reduced significantly compared with those of unoperated pulmonary arteries ($p < 0.05$).

Angiotensin I caused biphasic concentration-dependent contractions in all arteries with endothelium (Fig. 6). In arteries from rejecting dogs, contractions to angiotensin I in rings with endothelium decreased significantly compared with those from unoperated lungs; contractions increased with treatment of rejection. The sensitivity to angiotensin I was increased significantly in rings without endothelium from lungs treated for rejection compared with those of unoperated control lungs (ED_{50} − logM: 7.74 ± 0.23 and 7.05 ± 0.07 in the reversed and unoperated lungs, respectively).

Endothelin-1 caused concentration-dependent contractions in arterial rings with and without

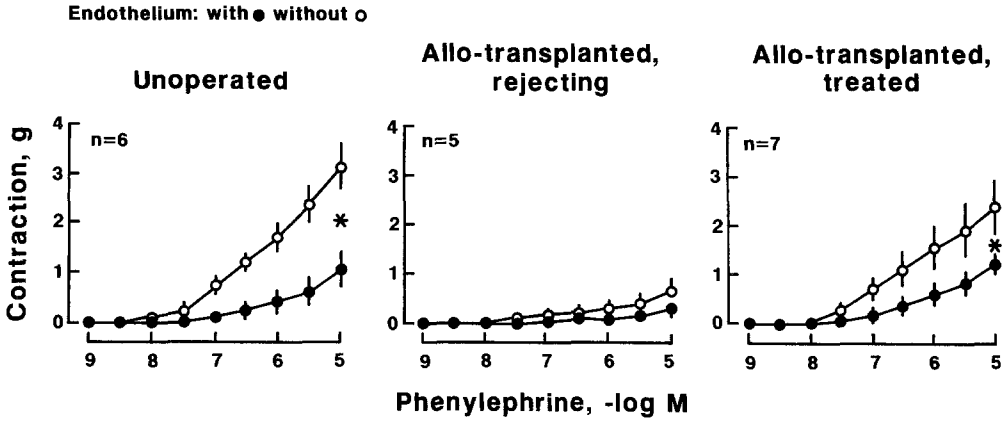

Fig. 1. Concentration-response curves to phenylephrine in canine pulmonary veins with and without endothelium. Unoperated dogs *(left panel)* and dogs with allotransplanted, rejecting *(middle panel)* and allotransplanted, treated *(right panel)* lungs. Data are expressed as grams increase in tension and are shown as means ± standard error of the mean. With rejection, contractions were reduced significantly in both rings with and rings without endothelium. Contractions of rings with and without endothelium increased significantly after treatment of rejection. (Analysis of variance, $p < 0.05$.)

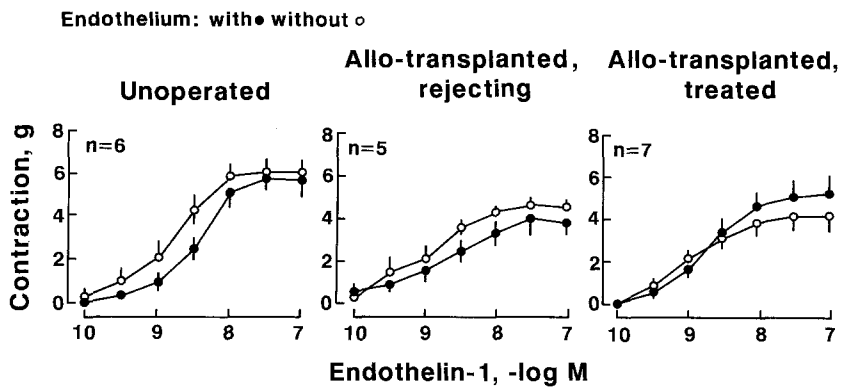

Fig. 2. Concentration-response curves to endothelin-1 in canine pulmonary veins with and without endothelium. Unoperated dogs *(left panel)* and dogs with allotransplanted, rejecting *(middle panel)* and allotransplanted, treated *(right panel)* lungs. Data are shown as means ± standard error of the mean and are expressed as grams increase in tension. The contractions were not affected significantly by either rejection or treatment.

endothelium. Contractions were not affected significantly by either rejection or treatment (data not shown, $n = 5$ and 6). Maximal tensions and sensitivity of the arteries to endothelin-1 were significantly less than those of the veins. Maximal tensions were 4.8 ± 0.6 gm and 6.0 ± 0.6 gm in arteries and veins without endothelium ($n = 6$ in each group), respectively. Sensitivity at ED_{50} (−logM) was 8.0 ± 0.2 and 8.9 ± 0.2 in unoperated arteries and veins without endothelium ($n = 6$ in each group), respectively.

In pulmonary arteries contracted with phenylephrine, histamine caused concentration-dependent relaxations (Fig. 7). These relaxations were greater in rings with endothelium compared with those without endothelium in unoperated arteries. Differences between relaxations of rings with and without endothelium were not observed in arterial rings from rejecting and treated dogs. In the rejecting group, maximal relaxation to histamine increased significantly both in rings with and in those without endothelium compared with relaxation of unoperated arteries (Fig. 7); with treat-

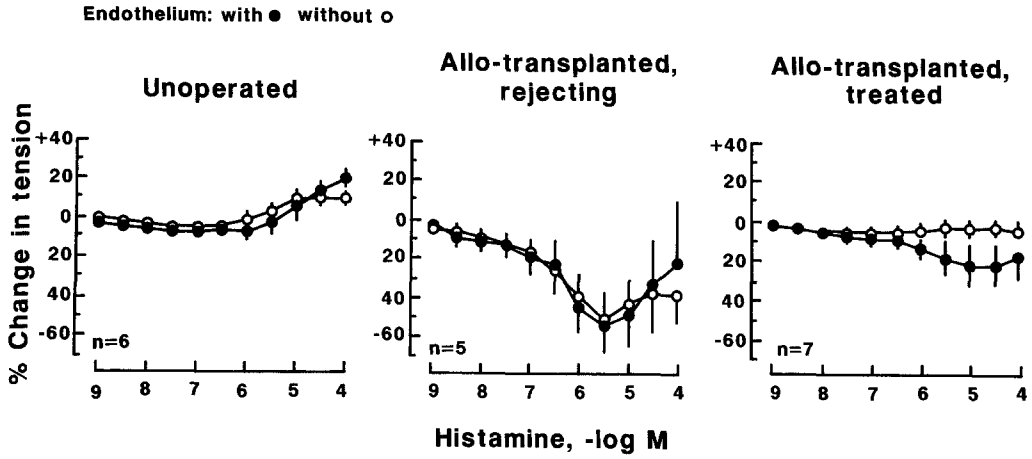

Fig. 3. Concentration-response curves to histamine in canine pulmonary veins with endothelium and without endothelium. Unoperated dogs *(left panel)* and dogs with allotransplanted, rejecting *(middle panel)* and allotransplanted, treated *(right panel)* lungs. Data are shown as means ± standard error of the mean and are expressed as a percent change in tension from a contraction to prostaglandin $F_{2\alpha}$ (2×10^{-6} mol/L). Relaxations were increased significantly with rejection. (Analysis of variance, $p < 0.05$.)

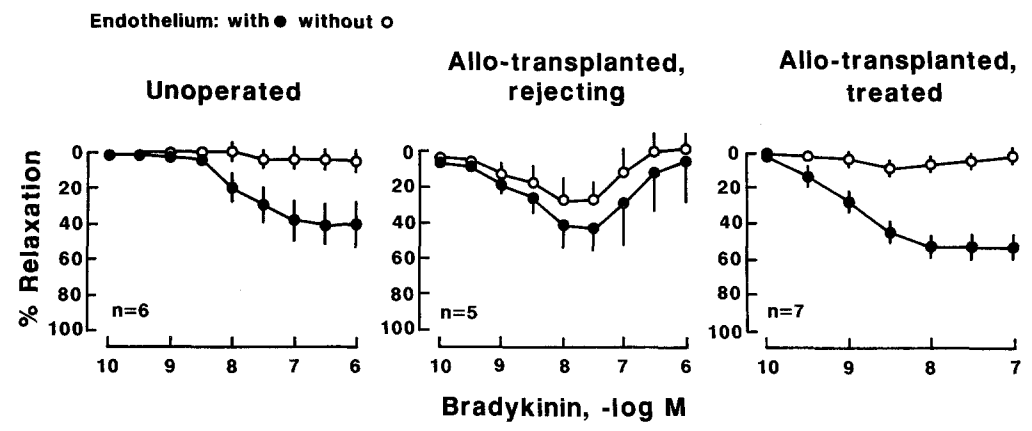

Fig. 4. Concentration-response curves to bradykinin in pulmonary veins with and without endothelium. Unoperated dogs *(left panel)* and dogs with allotransplanted, rejecting *(middle panel)* and allotransplanted, treated *(right panel)* lungs. Data are expressed as means ± standard error of the mean and are expressed as a percent change in tension from a contraction to prostaglandin $F_{2\alpha}$ (2×10^{-6} mol/L).

ment of rejection, relaxations were comparable with those of unoperated arteries (Fig. 7).

Bradykinin caused endothelium-dependent relaxations in pulmonary arteries from unoperated and treated dogs (Fig. 8). In arteries from rejecting dogs, relaxations were comparable in rings with and without endothelium. Maximal relaxations of the rings with endothelium were 62% to 58% less in arteries from rejecting dogs compared with the other groups.

As in the veins, both the calcium ionophore and nitric oxide caused concentration-dependent relaxations of arteries with and without endothelium, respectively. These were not altered significantly by either rejection or treatment of rejection (Table III).

Discussion

Two general conclusion can be drawn from the results of this study: (1) responses of the endothelium and smooth muscle of pulmonary veins are altered similarly to those of pulmonary arteries during episodes of acute rejection of lung allografts and (2) treatment of rejection that results in the

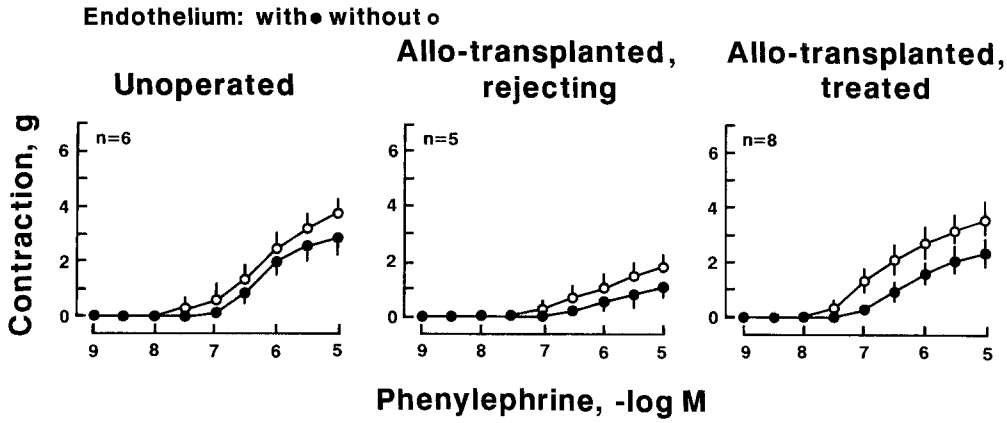

Fig. 5. Concentration-response curves to phenylephrine in canine pulmonary arteries with and without endothelium from unoperated dogs *(left panel)* and dogs with allotransplanted, rejecting *(middle panel)* and allotransplanted, treated *(right panel)* lungs. Data are expressed as grams increase in tension and are shown as means ± standard error of the mean. The contractions to phenylephrine were reduced significantly ($p < 0.05$) in arteries with endothelium from rejecting lungs compared with the controls, but this reduction was significant only in concentrations 10^{-5} and 3×10^{-5} mol/L in arteries without endothelium.

Table III. *Maximal responses and ED_{50} for relaxations to A23187 and nitric oxide in pulmonary arteries and veins from canine lungs*

	A23187		*Nitric oxide*	
	*Maximal relaxation** *(%) (3×10^{-7} mol/L)*	*ED_{50}†* (−logM)	*Maximal relaxation** *(%) (3×10^{-5} mol/L)*	*ED_{50}†* (−logM)
Arteries				
Unoperated ($n = 6$)	68.2 ± 9.8	7.2 ± 0.2	62.8 ± 5.0	6.3 ± 0.2
Allotransplanted, rejecting ($n = 4$)	58.4 ± 7.8	7.2 ± 0.1	51.9 ± 5.7	6.2 ± 0.1
Allotransplanted, treated ($n = 8$)	72.6 ± 5.3	7.3 ± 0.1	65.9 ± 9.8	6.3 ± 0.2
Veins				
Unoperated ($n = 6$)	36.4 ± 15.4	6.6 ± 0.1	68.4 ± 6.6	6.6 ± 0.1
Allotransplanted, rejecting ($n = 5$)	11.9 ± 1.3	6.4 ± 0.1	35.0 ± 9.9‡	6.4 ± 0.1
Allotransplanted, treated ($n = 7$)	29.6 ± 4.6	6.5 ± 0.1	59.5 ± 10.7	6.5 ± 0.1

All values are presented as means ± standard error of the mean; n = number of animals studied in each group.
*Relaxations expressed as percent decrease in tension from contractions to endothelin-1 (10^{-7} M)
†Concentration causing 50% of maximal relaxation
‡Statistically different from unoperated (analysis of variance, $p < 0.05$).

clinical end point of clearing of a chest roentgenogram restores the function of the blood vessel to an extent comparable with that found in arteries and veins from an unoperated lung.

Previous work from our laboratory has shown that in the immediate postoperative period (8 days) acute rejection alters responses of pulmonary arteries of the allotransplanted lung that are distinct from those found in autotransplanted lungs.[5] Therefore, changes are most likely due to the rejection process per se and not factors associated with the surgical intervention. The results of the present study confirm that rejection alters responses of pulmonary arteries of allotransplanted lungs and extends those observations to pulmonary veins. Changes in pulmonary venous smooth muscle and endothelium were found in response to neurotransmitters (phenylephrine), blood cell–derived substances (histamine), and endothelium-derived products (angiotensins, bradykinin, endothelin-1).

Pulmonary vasculature is innervated by adrenergic and cholinergic nerves.[10-12] Denervation caused by the transplantation process may result in denervation supersensitivity of adrenergic receptors.[13, 14] This would increase rather than inhibit contractions to phenyleph-

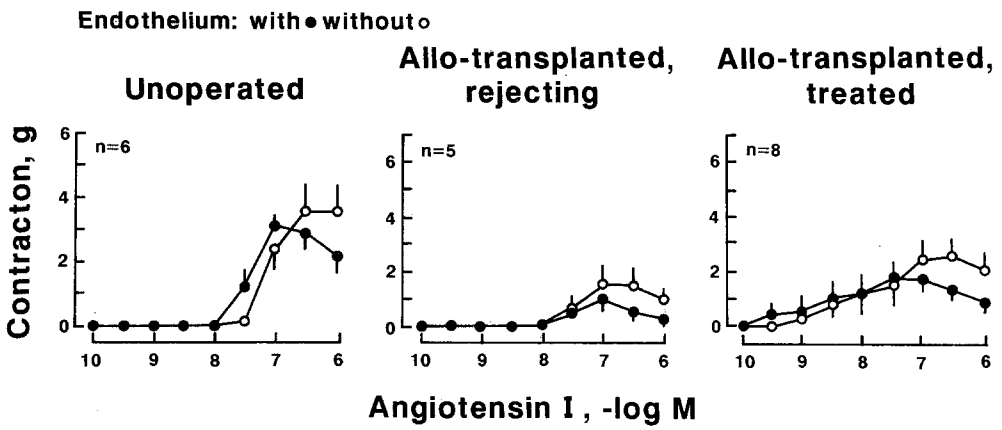

Fig. 6. Concentration-response curves to angiotensin I in canine pulmonary arteries with and without endothelium. Unoperated dogs *(left panel)* and dogs with allotransplanted, rejecting *(middle panel)* and allotransplanted, treated *(right panel)* lungs. Data are expressed as grams increase in tension and are shown as means ± standard error of the mean. With rejection, contractions to angiotensin I decreased significantly in rings with and without endothelium compared with the controls. The sensitivity to angiotensin I increased significantly in rings without endothelium from lungs treated for rejection compared with the controls (ED_{50} − logM; 7.74 ± 0.25 and 7.05 ± 0.02 in the treated and control groups, respectively).

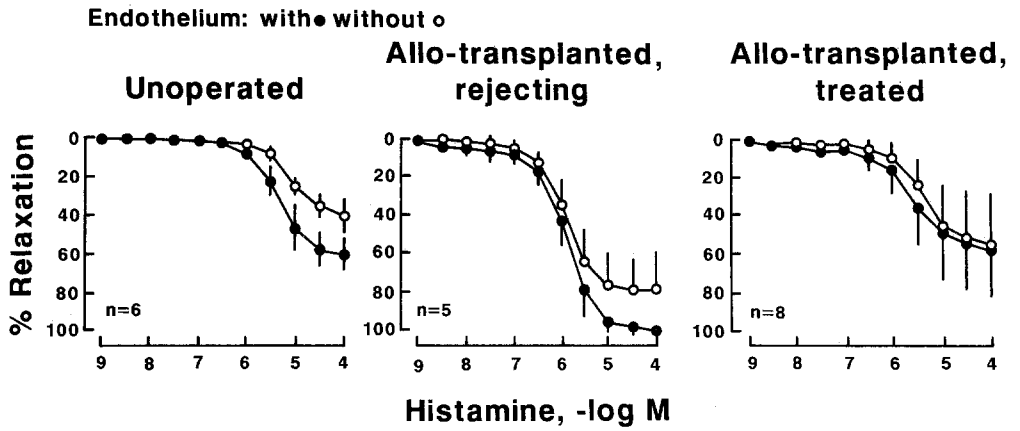

Fig. 7. Concentration-dependent relaxations to histamine in canine pulmonary arteries with and without endothelium. Unoperated dogs *(left panel)* and dogs with allotransplanted, rejecting *(middle panel)* and allotransplanted, treated *(right panel)* lungs. Data are shown as means ± standard error of the mean and are expressed as a percent change in tension from a contraction to phenylephrine (10^{-6} to 3×10^{-6} mol/L). In the rejecting group, maximal relaxations to histamine increased significantly (analysis of variance; $p < 0.05$) both in rings with and in those without endothelium compared with the controls. With treatment of rejection, the relaxations were comparable with those of control rings.

rine, as was observed in both pulmonary veins and arteries during rejection. Contractions of the blood vessels could be reduced by induction of nitric oxide in the smooth muscle by cytokines associated with the rejection process.[15-18] However, if this occurred, then one would expect that contractions to all agonists would be reduced. This was not the case. Contractions to phenylephrine were reduced, but not those to depolarization with potassium chloride (arteries) or receptor stimulation with endothelin-1 (arteries and veins). Therefore, these results suggest that the rejection process affects specific receptors or intracellular pathways for agonist activity.

Further support for the conclusion that rejection

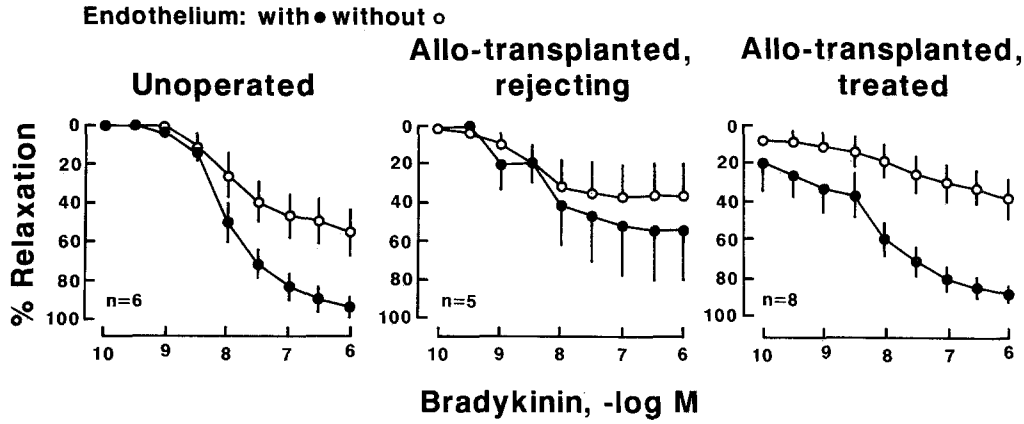

Fig. 8. Concentration-dependent responses to bradykinin in canine pulmonary arteries with and without endothelium. Unoperated dogs *(left panel)* and dogs with allotransplanted, rejecting *(middle panel)* and allotransplanted, treated *(right panel)* lungs. Data are shown as means ± standard error of the mean and are expressed as a percent change in tension from a contraction to phenylephrine (10^{-6} to 3×10^{-6} mol/L). Maximal relaxations were not statistically significant in the rejecting group compared with the other groups (55% ± 9.4%, 88% ± 3.9%, and 94% ± 1.1% in the rejected, treatment of rejection, and control group, respectively).

affects selective intracellular mechanisms is that relaxations to two receptor-activated relaxations are not affected the same way. During rejection, relaxations to histamine are increased in both pulmonary arteries and veins whereas those to bradykinin are reduced. Histamine is released by macrophages and can be produced by endothelial cells.[19] Histamine receptors may be coupled to guanine nucleotide regulatory proteins that inhibit cyclic adenosine monophosphate.[19, 20] Therefore, rejection may affect either the number or affinity of receptors for histamine or guanine nucleotide regulatory proteins. Increased relaxations of the smooth muscle (rings without endothelium) to histamine during rejection is consistent with increased capillary permeability and edema associated with opacification of the chest roentgenograms.

Bradykinin causes release of endothelium-derived relaxing factors, one of which is nitric oxide. Decreased relaxations to this agonist probably are not due to the inability of endothelial cells to produce relaxing factors inasmuch as relaxations to the calcium ionophore A23187 were not diminished during rejection. The ionophore releases relaxing factors by mechanisms that do not require receptor activation. In the arteries, then, decreased relaxations to bradykinin may be due to changes in receptor-activated release of relaxing factors.

In veins, relaxations to nitric oxide itself are reduced with rejection. Therefore, in the veins, rejection may affect enzyme systems like guanylate cyclase, which is activated by nitric oxide.

The mediators of and mechanisms by which arterial and venous responses were affected during rejection were not addressed in this study. However, these are probably multifactorial and should involve cell-cell interactions of leukocytes with the vessel wall and release of cytokines.[21-26] Large variability of reactions of the rings from different animals (for example, in response to bradykinin) and the variability in grades of rejection reflect that rejection is not a homogeneous process. Indeed, clinical rejection varies according to the immunologic compatibility of the donor and recipient.

Contractions to angiotensin I require its conversion to angiotensin II by the endothelial ectoenzyme angiotensin-converting enzyme.[27, 28] Activity of angiotensin-converting enzyme decreases with hypoxia[29] and circulating concentrations decrease during rejection of lung allografts [current study and reference 5]. Reduction of contractions of the pulmonary arteries with endothelium to angiotensin I during rejection suggests that surface enzymes are reduced with rejection. Interestingly, the pulmonary veins did not react to angiotensin I at all, an observation suggesting that the endothelium of pulmonary veins is not able to convert angiotensin I to angiotensin II or the smooth muscle of pulmonary veins does not have receptors for angiotensin II.

The second major conclusion of the current study is that treatment of rejection with pulsed steroids and immunosuppressive therapy reverses responses of pulmonary arteries and veins from the rejecting, transplanted lung to those observed in pulmonary arteries and veins from unoperated lungs. Changes in vascular responses with treatment is probably due to both methyprednisone and cyclosporine. Corticosteroids reduce inflammation and inhibit inducible nitric oxide synthase.[30] Cyclosporine was effective in limiting the rejection process as evidenced by clearing of the chest roentgenogram and improvement of the histologic grade of rejection. However, residual histologic rejection remained in the treated animals. Histologic grade of rejection is based on the number and type of infiltrating cells rather than their relative state of activation. Clinically, it often takes longer than this experiment allowed for complete histologic resolution of rejection. Therefore, it is possible for physiologic improvement of the rejection to precede movement of all cells from perivascular sites. The results of the current study support this, because pharmacologic responses of arteries and veins were different from those of the rejecting animals and similar to those from unoperated animals.

In conclusion, results of this study suggest that changes in responsiveness of pulmonary arteries and veins from acutely rejecting allotransplanted lungs are due to selective alterations of receptor-mediated processes in both the endothelium and smooth muscle. With treatment of rejection, these changes are reversible toward those observed in blood vessels from unoperated lungs.

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