sCR1sLe^X AMELIORATES ISCHEMIA/REPERFUSION INJURY IN EXPERIMENTAL LUNG TRANSPLANTATION

Uz Stammberger, MD^a
Jürg Hamacher, MD^b
Sven Hillinger, MD^c
Ralph A. Schmid, MD^a

Background: The nonspecific immune response with activation of the complement system and polymorphonuclear leukocytes is important for the mediation of reperfusion injury after lung transplantation. In this study, we investigated the combined blockade of the complement system and leukocyte adhesion by a novel drug combining soluble complement receptor type 1 (sCR1, CD35) with the selectin ligand sialyl Lewis X (sLe^X, CD15s) synthesized to sCR1sLe^X. Both sCR1 and sCR1sLe^X were supplied by AVANT Immunotherapeutics, Inc, Needham, Massachusetts.

Methods: Orthotopic allogeneic single left lung transplantation was performed in male rats (Brown Norway to Fischer F344; $n = 5$ in all groups) after a total ischemic time of 20 hours. Recipients received either no specific treatment (control) or administration of sCR1 (10 mg/kg) or sCR1sLe^X (10 mg/kg) 15 minutes before reperfusion by intracardiac injection. Twenty-four hours after reperfusion, the native contralateral lung was occluded to assess gas exchange of the graft only. In additional animals (5 per group), lung tissue was frozen 24 hours after reperfusion and assessed for myeloperoxidase activity as a measurement of neutrophil migration into the graft and thiobarbituric acid reactive substances to quantify lipid peroxidation.

Results: Graft function as assessed by arterial PO_2 in recipients treated with sCR1sLe^X was superior not only to that of controls (383 ± 53 vs 56 ± 7 mm Hg, $P = .000095$) but also to that of animals treated with sCR1 (243 ± 45 mm Hg, $P = .031$). This improvement was confirmed by significant reduction of neutrophil migration (0.33 ± 0.05 vs control, 1.0 ± 0.09 $\Delta OD/mg/min$, $P = .0000024$) and lipid peroxidation (6.2 ± 0.38 vs control, 10.6 ± 0.54 pmol/g, $P = .00021$).

Conclusions: Our data indicate that combined inhibition of complement activation and leukocyte adhesion with sCR1sLe^X reduces reperfusion injury significantly and that both mechanisms are effectively inhibited in this model. (J Thorac Cardiovasc Surg 2000;120:1078-84)

From the Division of General Thoracic Surgery,^a University Hospital, Berne, Switzerland, the Institute of Biochemical Pharmacology,^b University of Constance, Konstanz, Germany, and the Division of General Thoracic Surgery,^c University Hospital, Zurich, Switzerland.

Supported by a grant from the EMDO-Stiftung.

Read at the Eightieth Annual Meeting of The American Association for Thoracic Surgery, Toronto, Ontario, Canada, April 30–May 3, 2000.

Received for publication May 4, 2000; revisions requested Aug 9, 2000; revisions received Aug 11, 2000; accepted for publication Aug 29, 2000.

Address for reprints: R. A. Schmid, MD, Division of Thoracic Surgery, University Hospital, 3010 Berne, Switzerland (E-mail: ralph.schmid@insel.ch).

Copyright © 2000 by The American Association for Thoracic Surgery

0022-5223/2000 \$12.00 + 0 12/6/111175

doi:10.1067/mtc.2000.111175

Lung transplantation has become an established therapeutic option for end-stage pulmonary disease.¹ Ischemia/reperfusion injury remains the major problem in the early phase after lung transplantation. Improved preservation and flushing technique have reduced morbidity of early graft dysfunction; however, severe ischemia/reperfusion injury still occurs in 10% of lung transplant recipients.

The pathophysiology of this type of injury has been extensively studied. Therapeutic strategies blocking only one of the redundant pathways of the nonspecific immune response showed limited success.²⁻⁵ Therefore, combined treatment suppressing more than one of the mediators of ischemia/reperfusion injury seems more promising.

The complement system as a part of the humoral first line of defense and a primary mediator of the inflam-

matory process is one of the major pathways leading to severe graft dysfunction following reperfusion after prolonged ischemia. In post-transplantation lung ischemia/reperfusion, complement activation may accelerate tissue injury either directly by complement factors or indirectly by complement-mediated neutrophil activation.

A long series of studies demonstrates that neutrophils contribute to the vascular dysfunction and permeability in the initial phase after reperfusion. sCR1sLe^x (AVANT Immunotherapeutics, Inc, Needham, Mass) combines the effect of human soluble complement receptor type 1 (sCR1) and sialyl Lewis X (sLe^x) in one molecule. It blocks complement activation and reduces leukocyte-endothelial adhesion.⁶ In this study, the effect of sCR1sLe^x on post-transplantation graft function was evaluated in a small animal model of allogeneic single left lung transplantation after prolonged ischemia in comparison with untreated controls and recipients receiving sCR1 only.

Materials and methods

Weight-matched (200-250 g) male rats (donors: Brown Norway; recipients: Fischer F344) underwent orthotopic single left lung allotransplantation with a cuff technique used for the vessel anastomoses⁷ and a conventional running suture for the bronchial anastomosis.

All animals received humane care in compliance with the European Convention of Animal Care. The protocol was approved by the local animal study committee.

Donor procedure. Each animal was anesthetized by intraperitoneal administration of pentobarbital (50 mg/kg) and was heparinized (500 IU/kg). A tracheotomy was carried out and the lungs were ventilated through a cannula (Fio₂ = 1.0) by a Harvard rodent ventilator (Harvard Apparatus, South Natick, Mass) at a tidal volume of 10 mL/kg. After division of the inferior vena cava and resection of the left appendix of the heart, a small silicone tube was inserted into the main pulmonary artery. Both lungs were flushed with 20 mL of low-potassium dextran solution (Perfadex; Xvivo, Göteborg, Sweden)⁸ at a pressure of 20 cm H₂O. The trachea was tied in end-inspiration. The heart-lung block was removed and 14-gauge cuffs were placed around the pulmonary artery and vein. The vessels were inverted and tied onto the cuff. The lung was stored in low-potassium dextran solution at 4°C until implantation.

Recipient procedure. Transplantation was performed after 20 hours of cold ischemia (4°C). The recipient was anesthetized by breathing halothane in a glass chamber followed by intubation. Anesthesia was maintained with 2% halothane. A left lateral thoracotomy was performed in the fourth intercostal space. The left hilum was dissected. After the pulmonary artery and vein had been clamped with removable microclips, the pulmonary vein was opened, flushed with heparinized saline solution, and the cuff was inserted and fixed with 6-0 silk. In the same technique, the pulmonary

Table I. Warm ischemic time during implantation in all groups

	Groups	
	Arterial Po ₂ (min)	MPO, TBARS (min)
Control	20.8 ± 0.58	20.6 ± 0.8
sCR1	20.6 ± 0.51	20.6 ± 0.7
	<i>P</i> = .775 vs control	<i>P</i> = 1.0 vs control
sCR1sLe ^x	20.0 ± 0.32	19.4 ± 1.0
	<i>P</i> = .264 vs control	<i>P</i> = .283 vs control

artery was anastomosed. The native left lung was removed and the bronchial anastomosis performed with a running over-and-over stitch with 9-0 Monosof suture (Tyco Healthcare, Wollerau, Switzerland). The lung was first reventilated and then reperfused. A chest tube was inserted and the thoracotomy closed. The chest tube was removed after restoration of spontaneous breathing.

Assessment. The recipient animal was anesthetized by intraperitoneal administration of pentobarbital (50 mg/kg body weight).

Arterial blood gas analysis. Recipients were put to death 24 hours after reperfusion. Each animal was ventilated with an Fio₂ of 1.0, a frequency of 100 breaths/min, and a tidal volume of 8 mL/kg body weight by a tracheotomy. For functional assessment of the transplanted left lung, the right hilum was dissected and the right pulmonary artery and right main bronchus were occluded with microvessel clips. Five minutes after occlusion, a steady state was reached and an arterial blood gas sample was drawn from the thoracic aorta.

Myeloperoxidase activity (MPO) and thiobarbituric acid reactive substances (TBARS). Ventilation was the same as in donors. In these animals the lungs were flushed with 20 mL of saline solution through the pulmonary artery 24 hours after reperfusion. The transplanted lung was removed and snap-frozen in liquid nitrogen.

Study groups. In each group, 5 animals underwent transplantation for the evaluation of lung function by arterial blood gas analysis. In separate animals (n = 5/group), transplantation was done for the assessment of MPO and lipid peroxidation (TBARS).

Group I served as the control group, with no specific treatment. In group II recipients were treated with sCR1 (10 mg/kg), and in group III recipients received 10 mg/kg sCR1sLe^x. Both sCR1 and sCR1sLe^x were generously provided by AVANT Immunotherapeutics, Inc. Drugs were administered by intracardiac injection 15 minutes before reperfusion. For normal values of MPO and TBARS in rat lung, tissue samples of 4 animals were frozen directly after harvest.

Blood gas analysis. For assessment of arterial Po₂, a blood gas analyzer (AVL 993, AVL List GmbH, Graz, Austria) was used.

MPO assay. Lung samples were snap-frozen in liquid nitrogen and stored at -70°C until assay. Quantitative MPO activity was determined as previously described.⁹ The frozen lung tissue (100 mg) was homogenized in 1 mL of 0.5%

Fig 1. Arterial PO₂ (mm Hg) of the graft 24 hours after reperfusion (mean $\pm$ SEM).

Fig 2. Myeloperoxidase activity (Δ OD/mg/min) as measurement of neutrophil migration into the graft 24 hours after reperfusion (mean $\pm$ SEM).

hexadecyltrimethylammonium bromide, 5 mmol/L ethylenediaminetetraacetic acid, and 50 mmol/L potassium phosphate buffer (pH 6.2) with a tissue grinder. The homogenate was centrifuged at 10,000g for 15 minutes at 4°C. The supernatant was assayed for total soluble protein by the method of

Pierce Laboratories¹⁰ and for MPO activity. Enzyme activity was measured spectrophotometrically. Then, 10 mg of 5-fold supernatant was combined with 0.6 mL Hanks bovine serum albumin solution, 0.5 mL of 100 mmol/L potassium phosphate buffer (pH 6.2), 0.1 mL of 0.05% H₂O₂, and 0.1 mL of

Fig 3. TBARS levels (pmol/g) in allograft tissue as measurement of lipid peroxidation 24 hours after reperfusion (mean $\pm$ SEM).

1.25 mg/mL o-dianisidine. Color development was stopped by addition of 1% NaN_3 after either 5 or 20 minutes at room temperature. The optical density was measured at 460 nm with a spectrophotometer (Kadas 100; Dr Lange AG, Zurich, Switzerland). Color development from 5 to 20 minutes was linear. Enzyme activity is expressed as change in optical density unit per milligram of tissue protein per minute ($\Delta\text{OD}/\text{mg}/\text{min}$).

TBARS. Donor and recipient lung samples were frozen immediately and stored at -70°C until assay. TBARS were measured according to the method of Ohkawa, Ohishi, and Yagi¹¹ in 10% wet weight per volume homogenate. Aliquots (0.2 mL) of this homogenate were added to tubes containing 0.2 mL of 8.1% sodium dodecyl sulfate, 1.5 mL of 20% acetic acid solution adjusted to pH 3.5 with sodium hydroxide, and 1.5 mL of 0.8% solution of thiobarbituric acid. The mixture was brought to a volume of 4 mL by the addition of distilled water, heated at 95°C for 60 minutes, and then cooled with tap water. Next, 1 mL of distilled water and 5 mL of butanol/pyridine (15:1) were added (all chemicals by Fluka Chemie AG, Buchs, Switzerland). The solution was centrifuged at 4000 rpm for 10 minutes. The absorbance of the upper layer was measured at 532 nm with a spectrophotometer (Kadas 100; Dr Lange AG, Zurich, Switzerland). The TBARS levels were determined by reference to a standard curve of 1,1,3,3-tetramethoxypropane (Fluka Chemie AG, Buchs, Switzerland), and the results were expressed as picomoles of malondialdehyde per gram of wet lung.

Statistical analysis. All values are given as the mean $\pm$ standard error of the mean (SEM). Analysis of variance with planned comparison (contrast vectors) between the groups was applied. The STATISTICA 4.5 software (StatSoft, Tulsa, Okla) was used.

Results

Warm ischemic time in all transplantation groups was between 19.4 ± 1.0 and 20.8 ± 0.58 minutes, with no significant difference between groups (Table I).

Three recipients in the control group and 1 animal treated with sCR1 had severe edema with aspiration to the contralateral side and died within the first 3 hours after transplantation. One recipient in group III died of technical problems regarding the bronchial anastomosis. All these animals were excluded from analysis.

Blood gas analysis. Twenty-four hours after reperfusion, arterial PO_2 was very low in control animals (group I, 56 ± 7 mm Hg). Treatment with sCR1sLe^x resulted in better graft function than in controls (383 ± 53 mm Hg, $P = .000095$) (Fig 1). Administration of sCR1 improved gas exchange to a significantly lesser extent than sCR1 sLe^x (243 ± 45 mm Hg, $P = .0066$ vs control; $P = .031$ vs sCR1 sLe^x).

MPO assay. In grafts of recipients that did not receive any specific treatment, neutrophil migration as indicated by MPO assay was significantly elevated in

comparison with native lungs frozen immediately after harvest (1.03 ± 0.087 vs 0.22 ± 0.047 Δ OD/mg/min, $P = .000001$) (Fig 2). Treatment with sCR1sLe^x resulted in significant reduction of MPO activity (0.33 ± 0.051 Δ OD/mg/min, $P = .0000024$ vs control). In grafts treated with sCR1, a reduction of MPO activity to a lesser degree was noted (0.48 ± 0.074 Δ OD/mg/min, $P = .000036$ vs control; $P = .14$ vs sCR1sLe^x).

Interestingly, the difference between lung tissue frozen after harvest and grafts that received sCR1 was statistically significant ($P = .025$), whereas only a slight elevation of MPO activity was noted in group III (sCR1sLe^x, $P = .33$).

TBARS. Lipid peroxidation was significantly elevated in control animals 24 hours after reperfusion as compared with lung tissue assessed directly after harvest (10.65 ± 0.54 vs 3.94 ± 0.75 pmol/g, $P = .000005$) (Fig 3). Grafts of recipients treated with sCR1 revealed a distinct reduction of TBARS (8.32 ± 0.89 pmol/g, $P = .022$ vs control). Treatment with sCR1sLe^x reduced lipid peroxidation significantly not only compared with controls (6.23 ± 0.38 pmol/g, $P = .00021$) but also compared with recipients that received sCR1 ($P = .037$).

Discussion

In this model of unilateral left lung transplantation in rats after prolonged ischemia, sCR1sLe^x significantly improves gas exchange of the graft 24 hours after reperfusion compared with either untreated controls or recipients treated with sCR1. Reperfusion injury in lungs treated with sCR1sLe^x was significantly reduced, as substantiated by less neutrophil influx and reduction of tissue injury mediated by oxygen-derived free radicals. This study may indicate that the combined inhibition, in contrast to inhibition of a single proinflammatory pathway, is a promising strategy to reduce post-transplantation lung ischemia/reperfusion injury.

Complement is a proteolytic cascade system interacting with cells and is present in the blood plasma and other body fluids of all vertebrates. It mediates an inflammatory response by production of specific peptides with anaphylactic or chemotactic activity and the interaction with specific complement receptors on cell surfaces. The innate immune response is amplified by products of the complement components C3 and C5, which cause an increase in vascular permeability (C3a, C5a), leukocyte adhesion, and subsequent migration. Complement stimulates the synthesis of different proinflammatory cytokines. The final product of complement activation, the membrane attack complex, leads to direct membrane damage in target cells.

Complement receptor type 1 (CR1, CD35, C3b/C4b

receptor) is a transmembrane glycoprotein expressed on erythrocytes and virtually all leukocytes. On phagocytes, CR1 is involved in the initial binding of particles coated with activated complement component 3 (C3b), which are subsequently ingested. CR1 is not only a cellular receptor, but also the most potent inhibitor of both the classical and alternative pathways of complement activation. Activated neutrophils and macrophages shed the extracellular portion of CR1 (soluble CR1, sCR1).¹² It is more than 100-fold more effective than any other soluble complement regulatory proteins, for example, factor H, the physiologic cofactor for inactivation of C3b in plasma.^{13,14} Soluble CR1 accelerates the decay of the C3/C5 convertase complexes and participates in the degradation of C3b and C4b.¹³ Inhibition of complement activation by administration of sCR1 protects against lung injury in different models of acute inflammatory response.¹⁵ The inhibition of the nonspecific immune response after ischemia/reperfusion by administration of sCR1 in a model of myocardial infarction in rats demonstrated a beneficial effect.¹⁴ Administration of sCR1 during unilateral lung transplantation in swine after prolonged ischemia completely inhibited complement activity and significantly reduced reperfusion edema, indicating the important role of complement activation for the development of ischemia/reperfusion injury.¹⁶ The relevance of these findings has been confirmed recently by a clinical multicenter trial in patients undergoing lung transplantation.¹⁷

Complement mediates activation and migration of neutrophils by C5a. Therefore, complement exerts the post-transplantation reperfusion injury not only by its effector proteins but also by stimulation of polymorphonuclear leukocytes. Besides β -integrins and members of the immunoglobulin supergene family, selectins are adhesion molecules initiating leukocyte migration. These adhesion molecules are up-regulated in a number of different lung injuries. Specific inhibitors of neutrophil adhesion such as monoclonal antibodies and selectin ligands¹⁸ and inhibitors of neutrophil selectin up-regulation as leumedins have been shown to reduce post-transplantation lung reperfusion injury in animal studies. In a rat model, combined administration of monoclonal antibodies against intracellular adhesion molecule-1, CD11a, and CD18 resulted in superior gas exchange 24 hours after reperfusion and reduced accumulation of neutrophils in the lung tissue.¹⁹ Blockade of P-selectin by an anti-P-selectin immunoglobulin G antibody improved graft function and reduced polymorphonuclear leukocyte infiltration after rat lung transplantation.²⁰

The oligosaccharide sLe^x is a ligand common to all selectins and therefore an attractive compound for the inhibition of selectin-dependent neutrophil adhesion. In a canine model of left lung allotransplantation after prolonged ischemia, administration of the carbohydrate sLe^x analog CY-1503 improved the graft's gas exchange ($P < .01$) and reduced neutrophil migration as assessed by MPO assay and neutrophil count in bronchoalveolar lavage fluids.²¹

Recently, the glycoprotein product sCR1sLe^x has been synthesized by covalent post-translational modification of sCR1 with sLe^x glycosylation in a mammalian cell line.⁶ sCR1sLe^x maintains the complement blocking activity of sCR1, blocks P-selectin-mediated cellular adhesion, and binds to cell surface E-selectin in vitro. In vivo, its effects on experimental stroke and myocardial infarct size have been studied: Zacharowski and colleagues²² subjected Wistar rats to 30 minutes of ischemia of the left anterior descending coronary artery, followed by 2 hours of reperfusion. Treatment with either sCR1 or sCR1sLe^x resulted in reduction of infarct size and less release of troponin T from injured myocytes. There was no clear superiority of sCR1sLe^x as compared with sCR1 alone; however, a longer ischemic time and reperfusion period might have emphasized the trend to a better improvement. In experimental cerebral ischemia in mice, pretreatment with sCR1sLe^x reduced infarct volume up to 11-fold in a dose-dependent manner and decreased intracerebral hemorrhage and neurologic deficit 24 hours after stroke.²³ Infusion of sCR1 led to less reduction in infarct size and higher neurologic impairment. Interestingly, administration of both drugs after the onset of ischemia at the time of reperfusion also improved outcome, but to a lesser degree. Taking into account the clinical situation, in which treatment would probably be performed after the onset of reperfusion injury, further studies are needed to evaluate the effect of sCR1sLe^x in already established lung ischemia/reperfusion injury.

REFERENCES

- Williams TJ, Snell GI. Early and long-term functional outcomes in unilateral, bilateral, and living-related transplant recipients. *Clin Chest Med* 1997;18:245-57.
- Corcoran PC, Tse SS, Katz NM, Wang Y, St Louis JD, Foegh ML, et al. Reduction of conjugated dienes in lung transplantation: effect of BN 52021. *Ann Thorac Surg* 1993;56:1279-83.
- Ide S, Kawahara K, Takahashi T, Sasaki N, Shingu H, Nagayasu T, et al. Donor administration of PAF antagonist (TCV-309) enhances lung preservation. *Transplant Proc* 1995;27:570-3.
- Wahlers T, Hirt SW, Haverich A, Fieguth HG, Jurmann M, Borst HG. Future horizons of lung preservation by application of a platelet-activating factor antagonist compared with current clinical standards: Euro-Collins flush perfusion versus donor core cooling. *J Thorac Cardiovasc Surg* 1992;103:200-4.
- Mizutani H, Minamoto K, Aoe M, Yamashita M, Date H, Andou A, et al. Expression of endothelin-1 and effects of an endothelin receptor antagonist, TAK-044, at reperfusion after cold preservation in a canine lung transplantation model. *J Heart Lung Transplant* 1998;17:835-45.
- Rittershaus CW, Thomas LJ, Miller DP, Picard MD, Geoghegan-Barek KM, Scesney SM, et al. Recombinant glycoproteins that inhibit complement activation and also bind the selectin adhesion molecules. *J Biol Chem* 1999;274:11237-44.
- Mizuta T, Kawaguchi A, Nakahara K, Kawashima Y. Simplified rat lung transplantation using a cuff technique. *J Thorac Cardiovasc Surg* 1989;97:578-81.
- Steen S, Kimblad PO, Sjöberg T, Lindberg L, Ingemansson R, Massa G. Safe lung preservation for twenty-four hours with Perfadex. *Ann Thorac Surg* 1994;57:450-7.
- Schmid RA, Yamashita M, Ando K, Cooper JD, Patterson GA. Lidocaine reduces reperfusion injury and neutrophil migration in canine lung allografts. *Ann Thorac Surg* 1996;61:949-55.
- Smith PK, Krohn RI, Hermanson GT, Mallia AK, Gartner FH, Provenzano MD, et al. Measurement of protein using bicinchoninic acid. *Anal Biochem* 1985;150:768-85.
- Ohkawa H, Ohishi N, Yagi K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. *Anal Biochem* 1979;95:351-8.
- Hamacher J, Sadallah S, Schifferli JA, Villard J, Nicod LP. Soluble complement receptor type 1 (CD35) in bronchoalveolar lavage of inflammatory lung diseases. *Eur Respir J* 1998;11:112-9.
- Fearon DT. Anti-inflammatory and immunosuppressive effects of recombinant soluble complement receptors. *Clin Exp Immunol* 1991;86(Suppl 1):43-6.
- Weisman HF, Bartow T, Leppo MK, Marsh HC Jr, Carson GR, Concino MF, et al. Soluble human complement receptor type 1: in vivo inhibitor of complement suppressing post-ischemic myocardial inflammation and necrosis. *Science* 1990;249:146-51.
- Mulligan MS, Yeh CG, Rudolph AR, Ward PA. Protective effects of soluble CRI in complement- and neutrophil-mediated tissue injury. *J Immunol* 1992;148:1479-85.
- Schmid RA, Zollinger A, Singer T, Hillinger S, Leon-Wyss JR, Schob OM, et al. Effect of soluble complement receptor type 1 on reperfusion edema and neutrophil migration after lung allotransplantation in swine. *J Thorac Cardiovasc Surg* 1998;116:90-7.
- Zamora MR, Davis RD, Keshavjee SH, Schulman L, Levin J, Ryan U, et al. Complement inhibition attenuates human lung transplant reperfusion injury: a multicenter trial. *Chest* 1999;116:46S.
- Brandt M, Boeke K, Phillips ML, Steinhoff G, Haverich A. Effect of oligosaccharides on rejection and reperfusion injury after lung transplantation. *J Heart Lung Transplant* 1997;16:352-9.
- DeMeester SR, Molinari MA, Shiraishi T, Okabayashi K, Manchester JK, Wick MR, et al. Attenuation of rat lung isograft reperfusion injury with a combination of anti-ICAM-1 and anti-beta2 integrin monoclonal antibodies. *Transplantation* 1996;62:1477-85.
- Naka Y, Toda K, Kayano K, Oz MC, Pinsky DJ. Failure to express the P-selectin gene or P-selectin blockade confers early pulmonary protection after lung ischemia or transplantation. *Proc Natl Acad Sci U S A* 1997;94:757-61.
- Schmid RA, Yamashita M, Boasquevisque CH, Ando K, Fujino S,

Phillips L, et al. Carbohydrate selectin inhibitor CY-1503 reduces neutrophil migration and reperfusion injury in canine pulmonary allografts. *J Heart Lung Transplant* 1997;16:1054-61.

22. Zacharowski K, Otto M, Hafner G, Marsh HC Jr, Thiernemann C. Reduction of myocardial infarct size with sCR1sLex, an alternatively glycosylated form of human soluble complement receptor type 1 (sCR1), possessing sialyl Lewis x. *Br J Pharmacol* 1999;128:945-52.
23. Huang J, Kim LJ, Mealey R, Marsh HC Jr, Zhang Y, Tenner AJ, et al. Neuronal protection in stroke by an sLex-glycosylated complement inhibitory protein. *Science* 1999;285:595-9.

Discussion

Dr Robert J. Keenan (*Pittsburgh, Pa*). What if you were to pretreat your donors in an effort to inhibit some of the adhesion of donor leukocytes as opposed to the adhesion of recipient leukocytes after transplantation?

Dr Stammberger. That is probably a good idea, but we have not done that yet. Taking into account that the effect of combined blockade of not only neutrophil adhesion but also complement activation is tested, it seemed more appropriate to treat the recipients.

THE THORACIC SURGERY FOUNDATION FOR RESEARCH AND EDUCATION

Giving to Your Foundation Is Easy

Contributions to The Thoracic Surgery Foundation are tax deductible. For example, the real cost of a \$1000 contribution is only \$600 if you are in the 36% tax bracket. Cash gifts can be deducted to about 50% of your income.

Gifts of appreciated stock to The Foundation also offer appealing tax advantages. Securities which have appreciated significantly can be donated without capital gains taxation. Yet, the full fair market value of the securities is tax deductible. Consult your financial advisors about your personal circumstances with respect to both gifts of cash and securities.

Thoughtful estate planning is an excellent way to meet your financial and personal goals for yourself, your family, and your charities. The Foundation can assist with this planning on a confidential basis. Please contact Frank Kurtz at The Foundation's office at 312-644-6610, extension 3425 (telephone) or fkurtz@sba.com (E-mail).

Kennedy School Sabbatical

The Thoracic Surgery Foundation for Research and Education announces that applications for a year-long sabbatical to do graduate studies in health care policy at Harvard University's Kennedy School of Government are now available. Candidates will be accepted early on a rolling admission basis by the graduate school, and scholarships will be awarded by The Foundation's Education Committee. The Kennedy School will review completed applications for the program starting January 3, 2001, and is prepared to make decisions on applications within two weeks of their receipt. The Foundation will make a decision on an application for Alley-Sheridan Scholarship within two weeks of its receipt to The Foundation office. If an interested surgeon has applied to the Kennedy School very early in January, it is possible to have the admissions and scholarship decisions completed by February 1. Please call Lainie Castle at The Foundation office for more information at 312-644-6610, extension 4798.