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Beneficial Effects of Recombinant Soluble P-Selectin Glycoprotein Ligand (rPSGL-Ig) on Donor Brain Death-Related Early Changes and Late Outcome after Kidney Transplantation

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Systemic changes occurring after donor brain death (BD) may intensify early inflammatory processes in peripheral organs and accelerate acute allograft rejection after transplantation. Selectins are responsible for initial binding of circulating inflammatory cells to activated vascular endothelium. By competitive binding, a recombinant soluble selectin ligand (rPSGL-Ig) has been shown to inhibit their activity and to block the subsequent inflammatory cascade. In this study, the authors investigated both early and late effects of rPSGL-Ig in a chronic rejection rat renal allograft model. Six hours after induction of BD, kidneys from F344 donors were grafted into Lewis recipients. Kidneys from living donors (group 1) and normotensive BD-donors (group 2) served as controls. rPSGL-Ig was given intravenously (50 µg) to the donor animal 3 h after BD. A 2nd dose was given to the host after transplantation ($n = 8$, group 3). All recipients were treated with low-dose cyclosporin A (1.5 mg/kg, 10 d). Urinary protein levels (24-h urine) were measured serially, followed by histological analysis of the kidneys 200 days after transplantation. The mRNA transcription of representative inflammatory molecules was examined in untreated and treated BD donors before transplantation (6 h after BD) and after 200 days. Proteinuria in animals in groups 1 and 2, but not in group 3, increased progressively after approximately 12 weeks. Histologically, grafts from rPSGL-Ig treated animals showed only minor changes at 200 days, whereas the changes of chronic rejection had developed in untreated controls. Cytokine and chemokine activity, both early and late, was up-regulated in control kidneys, but not in those from the treated group. These data suggest that administration of rPSGL-Ig inhibits early inflammatory processes associated with donor BD. In turn, resultant chronic allograft dysfunction is prevented. These findings may be of particular clinical interest.

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Introduction

Clinical data have shown that kidney allografts from cadaver donors, as well as those with initial delayed function, experience more frequent episodes of acute rejection after transplantation.^{1,2}

This observation may be explained, at least in part, by experimental studies that suggest that the catastrophic central injury of donor brain death (BD) may initiate a systemic inflammatory response before organ removal, perfusion, and storage, which

in turn may trigger and amplify acute host immunologic activity after transplantation.³⁻⁶ The profound physiological and structural derangements in the peripheral organs include up-regulation of major histocompatibility antigens, adhesion molecules, cytokines, and other acute phase proteins. The early adhesion molecules, selectins, are responsible for initial endothelial binding of leukocytes to sites of inflammation. rPSGL-Ig, a recombinant soluble selectin ligand, inhibits selectin activity by competitive inhibition, preventing subsequent early diapedesis of inflammatory leukocyte cells into injured tissues. In this study, we investigated both early and late effects of rPSGL-Ig in a chronic rejection model using kidneys from living and BD donors.

Materials and Methods

Gradual onset BD was induced in F344 (RT1^{hl}) rats via inflation of an intracranial Fogarty catheter. Herniation of the brain stem was confirmed by flat-line EEG tracings with apnea, absent reflexes, and maximally dilated and fixed pupils. The animals were mechanically ventilated over a period of 6 h. The intra-arterial blood pressure was monitored continuously with a catheter inserted into the left femoral artery and connected via a transducer (Gould, P23ID, Gould, Inc, Cleveland, OH) to a blood pressure monitor (Recorder 2200S, Gould). Rats with a mean arterial blood pressure of < 80 mmHg sustained during the follow-up period were excluded from the study. The kidneys were then removed and grafted into Lewis (LEW) (RT1^l) recipients. Kidneys from living donors (LD, group 1) and normotensive BD-donors (group 2) served as controls. rPSGL-Ig (Genetics Institute/Wyeth Research, Cambridge, MA), consisting of the 1st extracellular 47 amino acids of the mature human PSGL-1 fused with a human IgG1Fc, was given intravenously (50 µg) to the donor 3 h after BD.^{7,8} A 2nd dose was administered to the recipient intravenously after revascularization of the kidney (*n* = 8, group 3). All recipients were treated with low-dose cyclosporin A (1.5 mg/kg, 10 d, Novartis Pharmaceuticals Corporation, E. Hanover, NJ). Kidney grafts were analyzed 6 h after BD and before transplantation by semiquantitative reverse transcriptase-polymerase chain reaction (RT-PCR)

for expression of the representative factors, IL-1β, MCP-1, TGF-β, TNF-α, and ICAM-1. Protein levels (24-h urine) were measured serially every 4 weeks. Histological analysis (hematoxylin and eosin, periodic acid-Schiff and trichrome) of the kidneys was carried out 200 days after transplantation. RNase protection assays were performed for representative cytokines occurring at 200 days using the RiboquantTM Multi-Probe RNase Protection assay system (BD PharMingen, San Diego, CA).

Results

RT-PCR analysis showed significantly up-regulated mRNA transcription (*P* < 0.0001) of ICAM-1, IL-1β, MCP-1, TNF-α, and TGF-β in untreated BD control kidneys (group 2) 6 h after induction of BD, and before transplantation. mRNA activity in kidneys of animals treated with rPSGL-Ig (group 3) remained at baseline and was comparable to naive controls. Urinary protein levels from animals in group 1 increased progressively after 90 days, those of group 2 recipients after 60 days. Proteinuria of treated group 3 rats remained at baseline. At 200 days, urine proteins in group 1 measured 191 ± 56 mg, group 2 = 278 ± 87 mg, and group 3 = 26 ± 11 mg (*P* < 0.001). Histologically, living donor kidneys in group 1 showed progressive changes of chronic rejection, with glomerulosclerosis (> 50%), tubular atrophy, intensified fibrosis, and focal interstitial mononuclear cell infiltration. These changes were markedly intensified in the kidney allografts from group 2, animals that were end-stage and were associated with increased mRNA expression of IL-1β, IL-2, TNF-β and IL-4. In contrast, cytokine activity was not expressed in kidneys from animals treated with rPSGL-Ig, resembling that of syngeneic controls.

Discussion

The state of donor BD causes intense inflammatory changes in the kidney and other peripheral organs and is thought to be an important antigen-independent risk factor for their subsequent transplantation. The 1st steps of this process involve the activation of vascular endothelium, with expression of a series of adhesion molecules, cytokines, and chemokines.^{3,9} P-selectin is one of the earliest acute phase proteins up-regulated and translocated to the

RECOMBINANT SOLUBLE P-SELECTIN GLYCOPROTEIN LIGAND (RPSGL-IG):

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plasma membrane of activated platelets and endothelial cells. PSGL-1 is a high-affinity counter-receptor for P- and E-selectin, is essential in the process of initial cell slowing and rolling, which is followed by firm adhesion and early transmigration of inflammatory leukocytes into injured tissues.¹⁰⁻¹² A recombinant soluble form of PSGL-1, rPSGL-Ig, inhibits selectin-mediated adhesion events through competitive binding and prevents the interaction between vascular endothelial cells, platelets, and polymorphonuclear leukocytes.¹³

The results of the present study demonstrate that administration of rPSGL-Ig down-regulates early nonspecific inflammatory events in kidneys from BD donors and abrogates subsequent induction of the immune responses. As a result, the kidney allografts remained functionally and morphologically intact over the long-term. The results suggest that treatment with rPSGL-Ig inhibits donor-BD-associated early inflammatory processes and, in turn, prevents resultant chronic allograft dysfunction. This strengthens the hypothesis that initial nonspecific injury to transplanted organs associated with donor BD, preservation, and storage may trigger or amplify host alloresponsiveness and may adversely influence short- and long-term outcome after transplantation.¹⁴ This new strategy may therefore be of particular interest for use in grafts of potentially diminished quality.

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REFERENCES

1. Koo DD, Welsh KI, McLaren AJ, et al. Cadaver versus living donor kidneys: impact of donor factors on antigen induction before transplantation. *Kidney Int* 1999;56:1551-9.
2. Troppmann C, Gillingham KJ, Benedetti E, et al. Delayed graft function, acute rejection, and outcome after cadaver renal transplantation. The multivariate analysis. *Transplantation* 1995;59:962-8.
3. Takada M, Nadeau KC, Hancock WW, et al. Effects of explosive brain death on cytokine activation of peripheral organs in the rat. *Transplantation* 1998;65:1533-42.
4. Pratschke J, Wilhelm MJ, Kusaka M, et al. Accelerated rejection of renal allografts from brain-dead donors. *Ann Surg* 2000;232:263-71.
5. Wilhelm MJ, Pratschke J, Beato F, et al. Activation of the heart by donor brain death accelerates acute rejection after transplantation. *Circulation* 2000;102:2426-33.
6. Kusaka M, Pratschke J, Wilhelm MJ, et al. Activation of inflammatory mediators in rat renal isografts by donor brain death. *Transplantation* 2000;69:405-10.
7. Kumar A, Villani MP, Patel UK, et al. Recombinant soluble form of PSGL-1 accelerates thrombolysis and prevents reocclusion in a porcine model. *Circulation* 1999;99:1363-69.
8. Khor SP, McCarthy K, DuPont M, et al. Pharmacokinetics, pharmacodynamics, allometry, and dose selection of rPSGL-Ig for phase I trial. *J Pharmacol Exp Ther* 2000;293:618-24.
9. van Der Hoeven JA, Ter Horst GJ, Molema G, et al. Effects of brain death and hemodynamic status on function and immunologic activation of the potential donor liver in the rat. *Ann Surg* 2000;232:804-13.
10. Somers WS, Tang J, Shaw GD, et al. Insights into the molecular basis of leukocyte tethering and rolling revealed by structures of P- and E-selectin bound to SLe(X) and PSGL-1. *Cell* 2000;103:467-79.
11. McEver RP, Beckstead JH, Moore KL, et al. GMP-140, a platelet alpha-granule membrane protein, is also synthesized by vascular endothelial cells and is localized in Weibel-Palade bodies. *J Clin Invest* 1989;84:92-9.
12. McEver RP, Cummings RD. Role of PSGL-1 binding to selectins in leukocyte recruitment. *J Clin Invest* 1997;100:S97-103.
13. Hayward R, Campbell B, Shin YK, et al. Recombinant soluble P-selectin glycoprotein ligand-1 protects against myocardial ischemic reperfusion injury in cats. *Cardiovasc Res* 1999;41:65-76.
14. Halloran PF. The emerging importance of the non-specific inflammatory response to injury in renal transplant outcomes. *J Nephrol* 1996;9:266.