Normalization of Brain Death—Induced Injury to Rat Renal Allografts by Recombinant Soluble P-Selectin Glycoprotein Ligand

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Abstract. Donor brain death has been considered a significant risk factor for both early and late organ allograft dysfunction. This central injury not only evokes an upsurge of catecholamines with resultant peripheral tissue vasoconstriction and ischemia but also promotes release of hormones and inflammatory mediators that may also affect the organs directly. One of the resultant influences of these events is the rapid upregulation of the acute-phase adhesion molecules, the selectins. These initiate leukocyte adhesion to vascular endothelium and trigger subsequent cellular and molecular changes in the compromised tissues. An established F344 → LEW rat model of chronic rejection was used to examine (1) whether the initial inflammatory events that develop within kidney allografts from brain-dead donors could be normalized using a recombinant soluble form of P-selectin glycoprotein ligand and (2) whether amelioration of these early changes would alter the inexorable progression of chronic allograft rejection. Untreated living donor controls experienced unrelenting chronic rejection over time. This complex process was accelerated in brain-dead donor kidneys. Treatment with P-selectin glycoprotein ligand prevented the early inflammatory changes in the transplanted organs and their subsequent (200 d) functional and morphologic manifestations, particularly when the soluble ligand was administered both to the donor before organ removal and to the recipient after engraftment. This strategy of using a naturally occurring selectin ligand to prevent donor-associated chronic graft dysfunction may be of special clinical interest in cadaver donor transplantation.

The clinical findings that kidneys from living unrelated donors perform as well over the short and long term as those from living related sources and that grafts from both donor groups are invariably superior to those from cadavers have emphasized that antigen-independent events, which include increased donor age and intercurrent disease, the state of brain death, and the period of ischemia, influence the quality of solid organs after transplantation and are important risk factors for their eventual outcome (1,2). Brain death, a central catastrophe unique to the cadaver organ donor, produces profound physiologic and structural derangements in the peripheral tissues of experimental animals both before and after placement in the recipient (3,4). These include massive upregulation of major histocompatibility antigens, adhesion molecules, cytokines, and other acute-phase proteins. Ischemia with systemic vasoconstriction is an important facet of brain death, secondary to the initial burst of catecholamines released into the circulation (5). The early injury and the associated reperfusion after transplantation evoke nonspecific inflammatory changes in the affected organs. However, additional factors may contribute to the peripheral effects of brain injury. Important changes in the dynamics of a series of hormones have been identified (6,7). Because brain death may also influence an organ differently than global ischemia alone, patterns of infiltration of circulating leukocytes through the tissues may also vary between the two conditions (8,9).

One result of these physiologic shifts is to cause prompt upregulation of selectins, cell-surface glycoproteins responsible for early recruitment of leukocytes to sites of injury (10,11). P-selectin is translocated within minutes from intracellular stores to the surface of vascular endothelial cells and/or platelets in response to inflammatory stimuli. E-selectin is then expressed on endothelial surfaces after transcriptional induction of its mRNA. These adhesion molecules react with their ligands on circulating polymorphonuclear leukocytes (PMN) to promote transient sticking (tethering) and slowing (rolling) along vessel walls. With stronger attachment to endothelium and diapedesis into the tissues via the sequential activity of other adhesion molecules, PMN become activated by locally produced chemokines and cytokines and trigger a further cascade of inflammatory/immunologic events.
A recombinant soluble form of P-selectin glycoprotein ligand (rPSGL-Ig) has been shown to inhibit the initial selectin activity and subsequent cellular and humoral events in global ischemia/reperfusion (12). In the present study, we examined its effects on kidney allografts from brain-dead (BD) donors, a putatively more complex model. Blockade of this initial step has allowed assessment of the influence of the agent on both early and late changes in long-surviving renal transplants in rats.

Materials and Methods

Animals and Operative Technique

Inbred male rats (Harlan Sprague-Dawley, Indianapolis, IN), 8 to 10 wk of age and weighing 200 to 250 g, were used throughout the experiments. Lewis rats (LEW, RT1<sup>l</sup>) served as recipients of renal isografts from LEW donors or allografts from Fisher (F344, RT1<sup>l</sup>) donors. The organs were flushed with 3 ml of cold lactated Ringers solution before removal and stored transiently in the cold solution (4°C) before heterotopic transplantation to the host abdominal great vessels. The left native kidney of the recipients was removed during the engraftment procedure and the right was removed after 10 d to provide time for the graft to recover from any brain death–associated and/or ischemic insult and to allow examination of the integrity of the ureteral anastomosis. All experiments were conducted in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and the Harvard Research Committee.

Model of Brain Death

F344 donor rats were anesthetized and tracheotomized. Brain death was induced by slow inflation (200 ± 250 mmHg) of a 3F Fogarty arterial embolectomy catheter (Baxter Healthcare, Irvine, CA) inserted into the subdural space via an occipital burr hole (3). The balloon was inflated over c. 15 min under continuous BP and electroencephalographic monitoring. Herniation of the brain stem was confirmed by flat-line tracings, plus the physical signs of apnea, absent reflexes, and maximally dilated and fixed pupils. The animals were then connected to a rodent respirator (Harvard Rodent Ventilator, model 683, Harvard Apparatus, South Natick, MA) and mechanically ventilated at a rate of 100 breaths/min with a tidal volume of 2.0 ml. Intra-arterial BP was monitored continuously with a PE 50 catheter inserted into the left femoral artery and connected via a transducer (P23ID, Gould, Cleveland, OH) to a BP monitor (Recorder 2200S, Gould). For avoiding peripheral effects of hypotension-associated ischemia, BD rats with a mean arterial BP <80 mmHg sustained during the follow-up period were excluded from the study. After 6 h, the kidneys were removed and transplanted.

Experimental Groups

Six groups (eight animals/group) were studied. Recipients of isologous kidneys from living donors (LD) served as syngeneic controls (LEW→LEW, group 1). LEW animals that received grafts from F344 LD (group 2) or BD F344 donors (group 3) served as allogeneic controls.

Several rPSGL-Ig treatment protocols were used in animals bearing kidney allografts from BD donors. In group 4 rats, the left kidney was perfused slowly (c. 70 to 90 s) in situ with 3 ml of cold Ringers solution 6 h after brain death via the clamped aortic segment, followed by installation of a solution of rPSGL-Ig (5 µg in 0.5 ml of phosphate-buffered saline [PBS]). An additional dose (5 µg in 0.3 ml of PBS) was administered after occlusion of the renal vein. As the ureter was also clamped to prevent any loss of material via the ureteric vessels, the solution was retained in the isolated kidney during the time needed to prepare the recipient (15 min). This protocol allowed rPSGL-Ig to inhibit P- and E-selectin expressed in the organ during the 6 h after donor brain death and during the brief period of cold ischemia (3).

Donors in group 5 received 3 h after induction of brain death an intravenous injection of 50 µg of rPSGL-Ig in 0.8 ml of PBS to diminish P- and E-selectin upregulation in the inflamed peripheral organs before their removal. In group 6 animals, a similar dose of rPSGL-Ig was injected intravenously into the donor 3 h after the central injury while a second dose of 50 µg was given to the transplant recipient intravenously at the time of unclamping the vessels after transplantation. Control kidneys (groups 1 to 3) were perfused with a nonspecific murine monoclonal antibody (L6-6 mAb, 50 µg in cold solution) 3 h after induction of brain death (Bristol Myers Squibb, courtesy of Dr. Robert Peach, Princeton, NJ). No control animals received rPSGL-Ig. Low-dose cyclosporine (Novartis Pharmaceuti
cals Corporation, E. Hanover, NJ) was administered to all allograft recipients in groups 2 to 6 (1.5 mg/kg/d × 10 d, subcutaneously) to inhibit early host immunologic activity and to allow long-term graft survival (13). Group 1 animals did not receive the agent as we have shown previously that it did not influence isograft behavior.

rPSGL-Ig

The cell surface glycoprotein adhesion molecule PSGL-1 is expressed by virtually all subsets of leukocytes and recognized by P-, E-, and L-selectins (14–16). The recombinant soluble form used in this study (Genetics Institute/Wyeth Research, Cambridge, MA) consists of the first extracellular 47 amino acids of mature human PSGL-1 fused with a human IgG1Fc (17). Two “hinge-proximal” amino acids at positions 234 and 237 within the IgG1Fc portion are mutated to alanine to reduce both complement activation and Fc receptor binding (18). The rPSGL-Ig molecule is secreted as a disulfide-bonded dimer with a half-life in rats of c. 100 h (19).

Physiologic Studies

Systolic BP was recorded at 2-wk intervals in representative recipients in groups 1, 2, 3, and 6 (n = 4 to 6 animals/group) using a tail cuff method. For determining functional changes occurring over time, urine (24 h) was collected every 4 wk from recipients in all groups (n = 8/group). Protein excretion was determined by measuring precipitation after interaction with 3% sulfosalicylic acid. Turbidity was assessed by absorbance at a wavelength of 595 nm using a Coleman Junior II Spectrophotometer. Serum creatinines were measured by a modified Jaffe’s reaction on an autoanalyzer (911; Hitachi, Indianap
ilis, IN).

Histology and Immunohistology

Representative portions of kidney grafts of groups 1, 2, 3, and 6 (n = 4 to 6/group) were fixed in 10% buffered formalin for histologic examination after 6 h of donor brain death and at 3 and 200 d after transplantation for immunohistology and molecular biology. Paraffin sections were evaluated using hematoxylin and eosin, periodic acid-Schiff (PAS), trichrome, and elastin stains. Additional pieces were quick frozen and stored at −80°C for immunohistology. Cryostat sections were fixed in paraformaldehyde-lysine-periodate to stain cell-surface antigens or in acetone for localization of cytokines using a peroxidase-antiperoxidase method as described (13). Monoclonal antibodies used for staining were purchased from Serotec (Harlan Bioproducts for Science, Indianapolis, IN), except where noted, and were directed against all rat leukocytes (CD 45, OX-1), T cells (T cell
receptor-α/β, R73), CD4 (W3/25) and CD8 (OX-8), B cells (CD45RA, OX-33), natural killer cells (CD161, 10/78), mononuclear phagocytes (CD68, ED1), and neutrophils (PMN, RP3, F. Sendo, Yamagata, Japan). Cell activation was assessed using mAb to rat MHC class II antigens (RT1B, OX-3), P-selectin (CD62P, BD PharMingen, San Diego, CA), and E-Selectin (CD62E, BBA-1; British Biotechnology, Cowley, UK). Cytokine expression was determined using mAb to rat interleukin-1β (IL-1β), IL-2 (1D10), IL-4 (OX-81), IL-10 (AS-4; R&D, Minneapolis, MN), tumor necrosis factor α (TNF-α; MAB 510, R&D), transforming growth factor β (TGF-β; AB-100-NA, R&D), and interferon-γ (IFN-γ; Δβ-10, P. van der Meide, Rijswijk, Holland). Control mAb and secondary antibodies were purchased from BD PharMingen. Isotype-matched mAb or purified IgG1 and controls for residual endogenous peroxidase activity were included in each experiment.

The numbers of labeled cells within 20 consecutive high-power fields (×40 magnification) were determined in three kidneys per group. Expression of cytokines and chemokines within these fields is reported on the basis of semiquantitative assessment.

**RNAse Protection Assays**

RNA protection was performed using the Riboquant Multi-Probe RNAse Protection assay system (BD PharMingen) to analyze a panel of cytokines relevant for initial nonspecific inflammatory events occurring in the organ (after 6 h before brain death) and after (3 d) engraftment and those associated with chronic rejection (CR) at 200 d. RNA was isolated from kidney grafts using Trizol. 32P-labeled probes were synthesized from the rCK-1 Multi-Probe Template Set (PharMingen) and were hybridized overnight with RNA samples in hybridization buffer according to the manufacturer’s instructions. Samples were digested with RNase and T1 mixed in RNase buffer, and protected probes were purified and run on a 5% acrylamide gel in 0.5% TBE buffer. Control RNA from kidney grafts and a dilution of the probe set (serving as size markers) were run in parallel. The gel was absorbed onto filter paper, dried, and exposed onto Kodak photographic paper at –70°C for 24 h. The RNA was analyzed by a phosphooimager using Imagequant software, allowing accurate quantification of mRNA.

**Reverse Transcriptase–PCR**

mRNA expression of representative adhesion molecules, chemotactants, and growth factors (intracellular adhesion molecule-1, monocyte chemotactic protein-1, and TGF-β) that were not measured by the RNase protection assay were analyzed together with IL-1β and TNF-α by reverse transcriptase–PCR (RT-PCR) in renal allografts from 6 h after induction of BD (n = 4 to 6/group) (3,4). Densities of competitive mimic and target gene DNA bands were measured by scanning densitometry using ScanJet 4c (Hewlett Packard, Corvallis, OR) with NIH Image software. The ratios of the densities of the respective bands were plotted to establish a linear relationship. Thus, absolute amounts of DNA from unknown samples were calculated from the known amount of the mimic in the starting reaction. Specimens were run in duplicate, and the average value was used. This assay is capable of detecting a twofold difference in target gene concentration and is as accurate as scintillation counting of radiolabeled PCR products (20). Results were expressed as a ratio to glyceraldehyde-3-phosphate dehydrogenase.

**Statistical Analyses**

Results are expressed as mean ± SEM. Each of the above mentioned studies was performed in eight rats per group. Comparisons were performed by analysis of variance or paired and unpaired t test when appropriate. Bonferroni’s correction for multiple comparisons was used to determine the level of significance. P < 0.05 was considered significant.

**Results**

**Physiologic Changes after BD and Transplantation**

With the gradual-onset brain death technique used in these studies, the systolic BP increased sharply over 15 to 25 min from a baseline mean arterial pressure of 98 ± 12 mmHg before injury to 214 ± 36 mmHg (n = 25, P < 0.0001). This gradually diminished to normotensive levels (90 to 110 mmHg) during the 6-h period before the kidney was removed for transplantation. Electroencephalographic monitoring showed flat-line tracings in BD animals versus physiologic activity in the controls.

All recipients survived during the 200-d observation. Systolic BP was assessed serially in representative animals throughout the follow-up period. Recipients of isografts in group 1 remained normotensive (110 ± 12 mmHg) with levels similar to those of ungrafted animals (n = 9). Recipients of LD allografts (group 2) varied in systolic BP between normal and c. 120 mmHg. That of rats bearing BD donor allografts, in contrast, increased progressively to 160 ± 15 mmHg by 20 wk (P < 0.001). Treated animals in group 6 were normotensive throughout the time of observation.

Renal function varied between the groups. Isografted animals (group 1) never manifested renal dysfunction (Figure 1). Proteinuria increased progressively in group 2 rats bearing chronically rejecting LD grafts after c. 12 wk. It became manifest earlier and reached higher levels in group 3 hosts with
grafts from BD donors. Urinary protein loss in recipients of BD donor kidneys perfused in situ with rPSGL-Ig (group 4) or from donors treated intravenously (group 5) was decreased and delayed in onset (P < 0.0001). When both donor and recipient were treated with rPSGL-Ig (group 6), renal function remained at baseline throughout the follow-up period.

As an additional assessment of graft function, levels of serum creatinine were monitored serially after transplantation in all groups (Figure 2). Creatinine was significantly increased in animals with kidneys from BD donors in group 3 at 200 d at levels consistently higher than in group 2 recipients of LD grafts (P < 0.0001). No increase in plasma creatinine levels occurred in treated animals in groups 4 to 6 over time, comparable to the syngeneic controls.

**Histology**

Except for minor tubular swelling, nongrafted kidneys in the BD animals seemed morphologically normal 6 h after the central injury. Isologous (group 1) and allogeneic LD (group 2) kidneys were also unchanged 3 d after transplantation. In contrast, allografts from BD donors (group 3) already showed severe tubular necrosis with widespread perivascular PMN and mononuclear cell infiltration. The kidneys of group 6 recipients showed only focal tubular necrosis at the corticomedullary junction and rare polymorphonuclear leukocyte (PMN) (Figure 3).

By 200 d after transplantation, group 2 kidneys showed progressive changes of CR, with widespread (>50%) glomerulosclerosis, obliteration of capillary loops, increased mesangial matrix, and marked mesangial cell proliferation (Figure 3). Tubular atrophy, fibrosis, and focal interstitial mononuclear cell infiltration were obvious. Kidney grafts from BD donor group 3 animals were end stage, with severe glomerulosclerosis (>90%), interstitial fibrosis, tubular atrophy and dilation, and dense cellular infiltration. In contrast, allografts in rPSGL-Ig–treated animals in group 6 resembled isografts (group 1), with <10% exhibiting minimal changes. These grafts were essentially normal.

**Immunohistology**

No immunohistologic changes were noted in any graft before transplantation, regardless of donor group. Infiltration of leukocyte populations was minimal after 3 d in kidneys of groups 1 and 2. Allografts in group 3 were infiltrated by relatively large numbers of PMN (c. 50%) and macrophages (c. 40%) (Table 1). Few lymphocytes were present. Cell populations infiltrating allografts of group 6 were sparse. Similarly, lymphocyte- and macrophage-associated cytokines were highly expressed in group 3 kidneys but absent in those of group 6 animals.

At 200 d, patterns of infiltration of leukocyte populations and their products generally mirrored the morphologic changes (Table 2). Cellular infiltration of group 1 isografts was minimal, and cell products were not expressed. The chronically rejecting LD allografts in group 2 showed a preponderance of macrophages (>75%) combined with c. 10% of each CD4+ and CD8+ T lymphocytes. PMN were absent. Interstitial and mononuclear cell staining for representative cytokines was moderate. Grafts from BD donors in group 3 showed increased numbers of macrophages and T cells versus group 2. Staining of interstitium and glomeruli with TGF-β was more intense in group 3 kidneys than in the other groups; >50% leukocytes were positive for TNF-α. In contrast, the grafts of rPSGL-Ig–treated animals (group 6) remained relatively bland, with minimal cell infiltration and features of immune activation resembling those in isografts.

**Molecular Changes**

As a corollary to the above findings, the kidneys in all animal groups were examined at intervals by RNAse protection assay and semiquantitative RT-PCR for mRNA levels of a representative series of lymphocyte- and macrophage-associated inflammatory mediators. Gene expression of all factors examined in the kidneys of untreated BD donor controls (group 3), except IL-1α, were upregulated significantly 6 h after the central injury and before transplantation (P < 0.0001), whereas levels in treated donors in group 6 remained at baseline, comparable to those from naive animals (Figures 4 and 5a). These cytokines remained upregulated in kidneys of group 3 at 3 d but were only minimally expressed in group 6 kidneys (Figure 5b). Those of groups 4 and 5 were also at baseline (data not shown).

The activity of cytokines at 200 d was clearly associated with the severity of the CR process as assessed morphologically (Figure 6). End-stage BD donor kidneys (group 3) expressed predominantly IL-1β, IL-2, TNF-β, and IL-4. In contrast, cytokines in kidneys from animals treated with rPSGL-Ig and without structural changes (group 6) resembled those of syngeneic controls. Expression in the other treated groups (groups 4 and 5) was in the same range (IL-1β and IL-5), minimally increased compared with kidneys of group 6 animals (IL-2 and IL-4), or upregulated marginally (TNF-β). This indicates that initial treatment of both the donor and the recipient, in particular, prevents significant expression of cytokines at a late period when the controls are experiencing overt changes of CR.
Figure 3. Histology of rat renal transplants (group 6) showing the effects of rPSGL-Ig treatment early and late after transplantation. (a and b) Periodic acid-Schiff–stained sections from day 3 allografts. Subsequent panels (c through f) show trichrome-stained sections of grafts harvested at day 200. Whereas allografts from group 3 at day 3 show widespread tubular vacuolization and sloughing in conjunction with a marked neutrophil-rich infiltrate (a), rPSGL-Ig therapy (group 6) led to minimal tubular injury and only minor focal mononuclear cell infiltration (b). At day 200, isografts (group 1) show some glomerular hypertrophy but are otherwise well preserved (c); allografts from LD (group 2) and much more intensified in those from BD donors (group 3) show advanced injury (d and e). In contrast, when both BD donors and recipients were treated with rPSGL-Ig (group 6), the allografts show almost normal architecture comparable to that of isograft controls. Sections are representative of four to six grafts/group/time point. Magnification, ×300.
Immunohistologic results of kidney allografts at day 3 after transplantation

<table>
<thead>
<tr>
<th></th>
<th>Group 1</th>
<th>Group 2</th>
<th>Group 3</th>
<th>Group 4</th>
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<tbody>
<tr>
<td>CD45+</td>
<td>4.3 ± 1.9</td>
<td>5.4 ± 1.4</td>
<td>28.7 ± 5.9</td>
<td>6.8 ± 2.2\textsuperscript{b}</td>
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<td>CD3+</td>
<td>0.8 ± 0.3</td>
<td>1.1 ± 0.5</td>
<td>4.8 ± 2.3</td>
<td>1.4 ± 0.8\textsuperscript{b}</td>
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<tr>
<td>MØ</td>
<td>3.7 ± 1.7</td>
<td>3.9 ± 1.9</td>
<td>11.2 ± 4.3</td>
<td>4.3 ± 1.8\textsuperscript{b}</td>
</tr>
<tr>
<td>PMN</td>
<td>0.1 ± 0.1</td>
<td>0.3 ± 0.1</td>
<td>14.3 ± 4.8</td>
<td>0.2 ± 0.1\textsuperscript{b}</td>
</tr>
<tr>
<td>IL-2R+</td>
<td>0.1 ± 0.1</td>
<td>0.1 ± 0.1</td>
<td>0.7 ± 0.5</td>
<td>0.1 ± 0.1\textsuperscript{b}</td>
</tr>
<tr>
<td>IFN-γ</td>
<td>Negative</td>
<td>Negative</td>
<td>Focal staining of 5% to 10% MNC</td>
<td>Negative</td>
</tr>
<tr>
<td>TNF-α</td>
<td>Negative</td>
<td>Negative</td>
<td>~20% MNC</td>
<td>Negative</td>
</tr>
<tr>
<td>TGF-β</td>
<td>Negative</td>
<td>Negative</td>
<td>Negative</td>
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\textsuperscript{a} Cells per high-power field. MNC, mononuclear cells.
\textsuperscript{b} \( P < 0.001 \) versus group 3.

Immunohistologic results of kidney allografts at day 200 after transplantation

<table>
<thead>
<tr>
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<th>Group 1</th>
<th>Group 2</th>
<th>Group 3</th>
<th>Group 4</th>
</tr>
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<tbody>
<tr>
<td>CD45+</td>
<td>4.3 ± 1.9</td>
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<td>47.4 ± 17.3</td>
<td>8.9 ± 1.9\textsuperscript{b,c}</td>
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<td>0.8 ± 0.3</td>
<td>6.8 ± 3.3</td>
<td>8.9 ± 4.8</td>
<td>1.6 ± 1.2\textsuperscript{b,c}</td>
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<tr>
<td>MØ</td>
<td>3.7 ± 1.7</td>
<td>14.8 ± 6.6</td>
<td>38.2 ± 11.7</td>
<td>6.3 ± 4.4\textsuperscript{b,c}</td>
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<tr>
<td>PMN</td>
<td>0.1 ± 0.1</td>
<td>1.1 ± 0.8</td>
<td>0.9 ± 0.5</td>
<td>0.1 ± 0.1\textsuperscript{b,c}</td>
</tr>
<tr>
<td>IL-2R+</td>
<td>0.1 ± 0.1</td>
<td>0.5 ± 0.2</td>
<td>1.8 ± 0.9</td>
<td>0.1 ± 0.1\textsuperscript{b,c}</td>
</tr>
<tr>
<td>IFN-γ</td>
<td>Negative</td>
<td>Focal staining of 5% to 10% MNC</td>
<td>Focal staining of 5% to 10% MNC</td>
<td>Negative</td>
</tr>
<tr>
<td>TNF-α</td>
<td>Negative</td>
<td>Focal staining of 5% to 10% MNC</td>
<td>&gt;50% leukocytes</td>
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<td>TGF-β</td>
<td>Negative</td>
<td>Moderate interstitial and glomerular staining</td>
<td>Dense interstitial and glomerular staining</td>
<td>Negative</td>
</tr>
</tbody>
</table>

\textsuperscript{a} Cells per high-power field.
\textsuperscript{b} \( P < 0.001 \) versus group 3.
\textsuperscript{c} \( P < 0.001 \) versus groups 2 and 3.

Discussion

Nonspecific peripheral injuries that occur secondary to donor brain death as well as those that surround organ removal, perfusion, and storage may initiate early renal dysfunction after transplantation. The associated inflammatory response, in turn, may trigger and amplify acute host immunologic activity against the graft (4). This observation would explain the apparent synergy noted clinically between the effects of delayed graft function and acute rejection episodes (1). At some later stage after resolution of the acute insults, the progression of chronic changes developing within a compromised allograft may become autonomous from any host-mediated processes, leading to its increasing deterioration and failure over time (21). Appreciation of the influence of these initial events on the quality of organs accepted for transplantation has stimulated examination of novel therapeutic approaches designed to normalize the affected tissue even before its placement in the recipient.

The relationship between donor brain death and inflammatory changes in peripheral organs is not fully defined. The autonomic storm produced from the central insult evokes autonomic changes in BP, with transient hypertension often followed by hypotension (5). As blood flow through a given tissue may not be uniform after brain death, the pattern of reperfusion may be different to that after the global ischemia associated with transplantation (9). The dynamics of the responsible catecholamines, well documented in model using large animals and in rats, may produce intense peripheral vasoconstriction and ischemia, with reduced redistribution of blood flow perfusion despite highly increased perfusion pressures (5,9). As vasodilation may then occur with decline in catecholamine levels and hypotension, low perfusion pressures may reduce regional blood flow further. This pattern seems different from the “low reflow phenomenon” in ischemic organs reperfused after transplantation, in which capillary swelling and platelet aggregation may impede blood flow despite persistent normotension. Resultant oxidative stresses may contribute to early organ dysfunction, as studied in models of ischemia but not after brain death (22–24).

Important endocrine changes that follow the central injury may also produce additional changes in peripheral organs that do not occur in pure ischemic models. As only 38% to 87% of cadaver
organ donors, for instance, develop diabetes insipidus from failure of vasopressin release from the hypothalamus and posterior pituitary gland, it is thought that viable cells in these structures may continue to function as long as 2 wk after death (25). Similarly, cells preserved in the periphery of the anterior pituitary, despite extensive central necrosis, may produce thyroid-stimulating hormone, prolactin, growth hormone, and other factors, as detected in BD patients 1 wk later (6,26). These substances have been implicated in metabolic injury to peripheral organs, although the subject still remains controversial.

Inflammatory factors are released into the circulation after a central insult or brain death. Clinically, expression of transcriptional levels of IL-1β and TNFα has been associated with episodes of focal cerebral ischemia, whereas IL-6 has been identified in the serum of BD patients (6,27). TNF-α, IL-1, IL-2, IL-6, and IL-10 have been found in high levels in the circulation of brain-injured and BD rats (3,28). Among the earliest events that develop after brain injury, as in other insults, is expression of a series of adhesion molecules in sequence by activated vascular endothelium (3,14,15). Upregulation of selectins mediates the adhesion of platelets, PMN, monocytes, and some lymphocytes to the vascular wall (29). The expression of P-selectin during the transplantation event is associated primarily with platelets, although immunohistologic assessment of these formed elements was not carried out in the present studies (30).

![Figure 4](image1.png)

**Figure 4.** mRNA expression of representative cytokines 6 h after induction of BD (group 3) was highly upregulated versus baseline values. Values of the rPSGL-Ig–treated group (group 6) were comparable to those of naive controls. Data are expressed as cytokine/glyceraldehyde-3-phosphate dehydrogenase ratio (n = 4 animals/group; *P < 0.0001 for each cytokine).

![Figure 5](image2.png)

**Figure 5.** Gene expression of a panel of factors assessed by RNAse protection assay was elevated in kidneys from BD donors (group 3) 6 h (A) and at 3 days after transplantation (B) after the central injury. These were essentially negative when the donor had been treated with rPSGL-Ig (group 6) (n = 4 animals/group, normalized optic density ratio; group 3 versus group 6, *P < 0.0001 for each cytokine).
rPSGL-Ig, a recombinant soluble form of PSGL-1, inhibits selectin-mediated adhesion events through competitive binding and prevents the interaction between vascular endothelial cells, platelets, and PMN (16, 17). Perfusion of an ischemic kidney with the agent dramatically reduces later acute cellular and molecular events associated with reperfusion, preventing resultant chronic changes over time (12). As brain death accelerates the inflammatory process, the timing of administration of rPSGL-Ig in these studies was designed to be relevant to the clinical situation by providing an adequate concentration of the blocking ligand during the period of injury surrounding transplantation. A low dose of rPSGL-Ig was added directly to the cold renal perfusate (group 4) or systemically to the donor 3 h after induction of brain death (group 5) to bind any P-selectin upregulated on the renal endothelium after brain death and during cold ischemia. This strategy was chosen as it had been shown previously that selectins are upregulated rapidly and that associated migration of PMN into the injured tissues begins while the organ is still in the host, well ahead of comparable cellular activity in LD grafts (3,4). As noted again in the present experiments, cell surface molecules and inflammatory mediators, already upregulated in the tissues 6 h after brain death, increase progressively with reperfusion and remain elevated for several days thereafter. Thus, in group 6 animals, an additional dose of rPSGL-Ig was given to recipients with revascularization of the transplant to extend the blockade of selectins during the initial days after reperfusion. As pharmacokinetic studies have determined that the half-life of rPSGL-Ig in normal rats is 100 h, this protocol also provided prolonged ischemia; it may also influence the sequelae of cold ischemia; it may also influence the sequelae of brain death (35). In addition, new immunosuppressive drugs such as rapamycin may reduce early inflammatory changes in transplanted organs. As more is learned about the complex sequelae of brain death injury, which seem more complex than those of ischemia alone, selective treatment of the organ, the donor, and/or the recipient may be given. Inhibition of one of the earliest steps, the upregulation of selectins, as described in these experiments, may be one such strategy.

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