Functional Role of Thromboxane Production by Acutely Rejecting Renal Allografts in Rats

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Abstract

We investigated the role of thromboxane in mediating the reduction in renal function and renal blood flow characteristic of acute renal allograft rejection. We transplanted kidneys from Lewis rats to Brown-Norway recipients. By the third day after transplantation, histologic changes that were consistent with cellular rejection occurred in the kidney. These changes were associated with a moderate reduction in renal function. By day 6, histologic changes of rejection were advanced and included interstitial and perivascular infiltration by mononuclear cells. The clearances of inulin and para-aminohippuric acid were also markedly reduced.

As renal function deteriorated, thromboxane B_2 (TXB_2) production by ex vivo perfused renal allografts increased progressively from 2 to 6 d after transplantation. However, prostaglandin (PG) E_2 and 6-keto $PGF_{1\alpha}$ production remained essentially unchanged. There was a significant inverse correlation between the in vivo clearance of inulin and the log of ex vivo TXB_2 production. Infusion of the thromboxane synthetase inhibitor UK-37248-01 into the renal artery of 3-d allografts significantly decreased urinary TXB_2 excretion and significantly increased renal blood flow (RBF) and glomerular filtration rate (GFR). Although renal function improved significantly after the acute administration of UK-37248-01, GFR and RBF did not exceed 33 and 58% of native control values, respectively. In other animals, daily treatment with cyclophosphamide improved the clearances of inulin and para-aminohippuric acid and reduced thromboxane production by 6-d renal allografts. These studies demonstrate that histologic evidence of rejection is associated with increased renal thromboxane production. Inhibition of thromboxane synthetase improves renal function in 3-d allografts. Cytotoxic therapy improves renal function, reduces mononuclear cell infiltration, and decreases allograft thromboxane production. Thus, the potent vasoconstrictor thromboxane A_2 may play a role in the impairment of renal function and renal blood flow during acute allograft rejection.

Introduction

The process of cellular rejection markedly reduces renal blood flow (RBF)[1] and impairs excretory function in renal allografts. Several investigators have described a reduction in renal cortical blood flow during rejection that is greater than expected based on the degree of morphologic changes in the kidney (1–4). Hollenberg et al. (3) suggested that infiltrating cells may produce local vasoconstrictors that could impair renal function. Because leukocytes and various renal cells produce locally vasoactive eicosanoids, alterations in prostaglandin (PG) and thromboxane (TX) metabolism may play an important role in renal allograft rejection. For example, Foegh et al. (5, 6) reported that urinary immunoreactive thromboxane B_2 (TXB_2) increases during episodes of acute rejection in human renal allograft recipients. However, the source of the TXB_2 that is excreted in urine and the physiologic significance of increased urinary TX is currently unknown (7–9).

Our objectives in this study were to evaluate the relationship between renal function in vivo and TXB_2 production in vitro, and to examine whether histologic evidence of acute cellular rejection correlated with increased TX production. To accomplish these objectives, we developed a model of acute renal allograft rejection characterized by a predictable temporal progression. Using a combination of ex vivo renal perfusion studies, histopathologic evidence, and in vivo clearance and hemodynamic studies with both a cytolytic agent and a TX synthetase inhibitor, we have characterized changes in renal function and renal eicosanoid production in this experimental model of acute renal allograft rejection.

Methods

Rat renal transplants. Renal allografts from male Lewis rats weighing 200–300 g were transplanted into female Brown-Norway rats weighing 150–190 g using the technique described by Fabre et al. (10). The left donor kidney of Lewis rats was prepared by freeing the ureter and bladder from all peritoneal attachments. Renal artery and aorta were separated from renal vein and inferior vena cava by blunt dissection. Small arteries not involved in renal circulation were ligated and cut. We infused heparin (1,000 U/kg, Upjohn Co., Kalamazoo, MI) intravenously and then clamped the donor aorta above the left renal artery. The donor kidney was flushed with 20 ml of an iced solution containing 10% mannitol, chloramphenicol (sodium succinate salt; Merrel-National Laboratories, Cincinnati, OH) and 100 U of heparin in half-normal saline. The kidney, ureter, and bladder were removed en bloc, including the renal artery with a 3-mm aortic cuff, and the renal vein with a 3-mm vena caval cuff. The kidney was placed into an iced solution of 10% mannitol in saline.

We prepared the recipients by carefully separating aorta and vena cava between the origin of the renal vessels and the bifurcation of the iliacs. An anastomosis was created between the recipient's aorta and the donor's aortic cuff; the recipient's vena cava was anastomosed to the donor vena caval cuff. Total ischemic time averaged 40 min.

Dr. Coffman performed these studies as a National Kidney Foundation Fellow.

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1. *Abbreviations used in this paper:* C_{In}, clearance of inulin; C_{PAH}, clearance of p-aminohippuric acid; GFR, glomerular filtration rate; PAH, p-aminohippuric acid; PG, prostaglandin; PGE_2, prostaglandin E_2; RBF, renal blood flow; TX, thromboxane; TXB_2, thromboxane B_2.

Donor and recipient bladders were attached dome to dome. The recipients' native kidneys were left in situ. Surgical mortality of the recipients was <10%.

Clearance studies. We measured clearances of inulin (C_{In}) and p-aminohippuric acid (C_{PAH}) on days 1 through 6 after transplantation in 32 animals. Animals were anesthetized with inactin, and a polyethelene catheter (PE-240) was inserted into the trachea to facilitate spontaneous respiration. The right carotid artery was cannulated to permit periodic sampling of arterial blood and to measure arterial blood pressure (Gould-Statham strain gauge; Gould Inc., Hato Rey, PR). The right jugular vein was cannulated to infuse carboxyl-^{14}C-inulin and glycyl-[^{3}H]PAH (New England Nuclear, Boston, MA) in a solution of 5% mannitol and 0.45% saline at a rate of 0.025 ml · 100 $g^{-1} \cdot min^{-1}$. Allograft and right native kidney ureters were cannulated to measure individual renal function. After a 1-h equilibration period, C_{In} and C_{PAH} were measured during two 30-min urine collections. Arterial blood samples were obtained at the midpoint of each collection.

Ex vivo perfusion. After completing in vivo clearance studies, we removed allografts for ex vivo perfusion to assess renal eicosanoid production in 21 animals. In addition to these transplanted kidneys, we perfused nontransplanted kidneys from normal Lewis rats ex vivo (n = 4) and nontransplanted kidneys of Lewis rats subjected to 45 min of renal pedicle cross-clamping. Using a modification of previously described methods (11), a polished metal perfusion catheter was inserted into the left femoral artery and advanced to the transplant anastomosis. The aorta was then ligated around the catheter. The renal vein of the allograft was cannulated with a flared PE-90 catheter. The kidney was removed and placed into a warming chamber (37°C).

We perfused kidneys with a cell-free Krebs-Henseleit buffered solution to avoid prostanoid contamination from nonrenal cells. Perfusate was warmed to 32°C and oxygenated (95% O_2, 5% CO_2) to meet, more adequately, metabolic demands of the ex vivo perfused kidney (12). In other studies, we have demonstrated that morphologic evidence of cellular necrosis is absent with perfusate warmed to 32°C and that there is extensive cortical necrosis when perfusate is delivered at 37°C. At 32°C, oxygen consumption by tubule suspensions from 4-h perfused kidneys is normal and there is no increase in renal venous lactate dehydrogenase activity during perfusion. Perfusate was delivered at a rate sufficient to maintain pressure between 100 and 120 mmHg (8 ml/min).

We collected two control samples of renal venous effluent during the first 30 min of ex vivo perfusion. We then stimulated prostanoid production by the kidney with 1-μg boluses of bradykinin given every 30 min (11, 13). Samples of renal venous effluent were collected on ice at 30-min intervals for a total of 4 h and 5-ml aliquots were stored at −70°C. Samples were thawed, extracted, and then assayed for TXB_2, prostaglandin E_2 (PGE_2), and 6-keto-PGF_1 by radioimmunoassay.

Cyclophosphamide treatment. In a second group of animals, we evaluated the effects of immunosuppression on renal allograft function and eicosanoid production. After transplantation, five rats were treated with the alkylating agent cyclophosphamide 20 mg · $kg^{-1} \cdot day^{-1}$ (Mead Johnson, Evansville, IN). The first dose was injected intravenously during transplantation, and subsequent daily doses were injected intraperitoneally. We performed renal clearance and ex vivo perfusion studies (as described above) in renal allografts after 6 d of cyclophosphamide therapy.

Effects of TX synthetase inhibition on renal hemodynamics. In a group of seven animals, we evaluated the effects of increased TX production on in vivo renal function in 3-d allografts. Renal clearance studies were performed and RBF was measured in native and donor kidneys before and after administering the TX synthetase inhibitor (14) UK-37248-01 (4-(2-[IH-imidazol-1-Y^1]ethoxy)benzoic acid HCl, Pfizer Inc., New York, NY. In these animals, a tapered PE-10 catheter was inserted into the femoral artery and advanced into the allograft's renal artery cuff (15). Normal saline or a solution of UK-37248-01 in saline was infused into the renal artery at a constant rate of 24 μl/min. The renal vein of the allograft was catheterized with a curved 0.965 mm o.d. teflon catheter (Bradford Scientific, Marble Head, MA) to collect samples for the determination of renal venous p-aminohippuric acid (PAH) concentration. RBF was calculated as: $C_{PAH}/[E_{PAH} \times (1\text{-Hct})]$ (E_{PAH}, PAH extraction; Hct, hematocrit).

After a 45-min equilibration period, we measured C_{In}, C_{PAH}, and E_{PAH} in the allograft and right native kidneys during two 30-min control periods. During control periods, normal saline was infused into the renal artery of 3-d allografts. These values were remeasured during two 30-min experimental periods while infusing the TX synthetase inhibitor UK-37248-01 (100 $\mu g \cdot kg^{-1} \cdot min^{-1}$). This dose was selected after performing in vivo dose response curves in preliminary renal function studies in 3-d allografts.

In a separate group of four animals with 3-d allografts, we documented that UK-37248-01 inhibited in vivo TXB_2 production. In these animals, the ureter of the transplanted kidney was cannulated and urine was collected on ice before and after UK-37248-01 infusion (100 $\mu g \cdot kg^{-1} \cdot min^{-1}$). Urine samples were extracted as described above and TXB_2 was measured by radioimmunoassay and expressed as picograms of TXB_2 produced per minute. In other studies using UK-37248-01 in hydronephrotic rats, we have found that 100 $\mu g \cdot kg^{-1} \cdot min^{-1}$ administered into the renal artery inhibits renal TXB_2 production ex vivo, reduces urinary TXB_2 excretion in vivo, and improves C_{In} and RBF in vivo after 24 h of unilateral ureteral obstruction.

Histologic studies. After renal clearance or ex vivo perfusion studies, all kidneys were fixed in buffered formalin for histologic evaluation. 3-μm sections were prepared and stained with hematoxylin and eosin. Both native and donor kidneys were examined for the presence of cellular infiltrates on days 1 through 6 after transplantation.

Analytical techniques. Tritium and ^{14}C radioactivity were measured in plasma and urine with a dual channel liquid scintillation system (Iso-Cap 300; Nuclear Chicago-TM Analytic Inc., Elk Grove Village, IL). Plasma and urine Na^+ and K^+ were analyzed with a lithium internal standard flame photometer (Instrumentation Laboratory, Inc., Lexington, MA), and U_{Osm} and P_{Osm} with a vapor pressure osmometer (Wescor Inc., Logan, UT).

Radioimmunoassays (RIAs). Prostanoids were extracted by passing samples of renal venous effluent through C_{18} columns (Sep-Pak C_{18} cartridges; Waters Associates, Millford, MA). Cartridges were then washed with distilled water, and samples were eluted with acetonitrile and dried at 35°C with dry nitrogen. Dried samples were resuspended in buffer and assayed for TXB_2, PGE_2, or 6-keto $PGF_{1\alpha}$ by RIA. Small amounts of tritiated compound were added to each sample before extraction and RIA to correct for recovery. Using this extraction procedure, 60–70% of [^{3}H]TXB_2, 70–80% of [^{3}H]6-keto-$PGF_{1\alpha}$, and 70–80% of [^{3}H]PGE_2 were recovered. All RIA reagents were purchased from Seragen Inc., Boston, MA. Specific antisera were generated in rabbits using immunogen complexed to bovine serum albumin. Antibody to TXB_2 demonstrated 50% binding at a final titer of 1:35,000; cross-reactivity to 2–3 dinor TXB_2 was 60%, and to other prostanoids was <0.1%. Sensitivity of the antibody (50% displacement of radioligand) was 12 pg. Antibody to PGE_2 demonstrated 50% binding at a final titer of 1:14,000; cross-reactivity was 6% for PGA_2, 3% for PGA_1, 1.3% for $PGF_{2\alpha}$, and <1% to other prostanoids. Sensitivity of the PGE_2 antibody was 73 pg. Antibody to 6-keto-$PGF_{1\alpha}$ demonstrated 50% binding at a final assay titer of 1:45,000; cross-reactivity was 7.8% for $PGF_{1\alpha}$, 6.8% for 6-keto-PGE_1, 2.2% for $PGF_{2\alpha}$, 0.7% for PGE_1, 0.6% to PGE_2, and <0.01% for the other prostanoids. Sensitivity of the 6-keto $PGF_{1\alpha}$ antibody was 47 pg.

Samples and standards were incubated with a mixture of antisera and a known amount of tritiated standard at 4°C for 20 h. Specific activity of the ^{3}H standards ranged between 110 and 165 Ci/mM. After incubation, we adsorbed free prostanoid with dextran-coated charcoal and measured ^{3}H remaining in the supernatant with a liquid scintillation counter. The unknowns, corrected for ^{3}H recovery from the extraction procedure, were compared with the standard curve in which the logarithm of the concentration was plotted against the logit of the B/B_0 value. The results are expressed as picograms per minute of prostanoid.

Statistics. Data are presented as means±standard error. Statistical

significance was assessed between groups with an unpaired t test, and within groups with a paired t test. The correlation between the log of TXB_2 produced ex vivo and C_{In} measured in vivo was determined by linear regression analysis.

Results

The clearances of inulin and PAH by native kidneys and allografts on days 1 through 6 after transplantation are depicted in Fig. 1. Native kidney C_{In} and C_{PAH} remained unchanged throughout most of the 6 d after transplantation with the exception of day 2. On day 2, both C_{In} and C_{PAH} increased slightly but not significantly above basal values. On the first day after transplantation, C_{In} and C_{PAH} of renal allografts were significantly reduced compared with native kidneys. On the second day after transplantation, function of the allografts improved and C_{PAH} was not different from that of the native kidneys (7.82 ± 1.82 ml·min^{-1}·kg^{-1} vs. 9.69 ± 1.45 ml·min^{-1}·kg^{-1}). Allograft C_{In} also improved but was still less than that of native kidneys (2.35 ± 0.45 ml·min^{-1}·kg^{-1} vs. 4.20 ± 0.48 ml·min^{-1}·kg^{-1}; $P < 0.025$). Allograft function declined progressively from days 3 through 6; native kidney function remained unchanged. On day 3, allograft C_{In} was significantly reduced when compared with day 2 (0.9 ± 0.3 vs. 2.35 ± 0.45 ml·min^{-1}·kg^{-1}, $P < 0.025$). C_{PAH} was also significantly reduced from 7.82 ± 1.26 ml·min^{-1}·kg^{-1} on day 2 to 2.91 ± 0.88 ml·min^{-1}·kg^{-1} on day 3 ($P < 0.025$). By day 6, C_{In} and C_{PAH} of donor kidneys decreased to 0.04 ± 0.04 and 0.24 ± 0.21 ml·min^{-1}·kg^{-1}, respectively ($P < 0.005$ vs. day 3 values).

Histologic examination of allografts on the first day after transplantation demonstrated patchy acute tubular necrosis with no evidence of acute cellular rejection. There were focal areas of brush border loss, sloughed epithelial cells, and occasional intraluminal casts. On day 2, tubules and glomeruli appeared histologically normal and there was only minimal cellular infiltration. However, on the third day after transplantation, there was marked infiltration of the interstitium by inflammatory cells, especially in perivascular areas, with sparing of glomeruli. 6 d after transplantation, there was marked progression of mononuclear cell infiltration throughout the interstitium of the allograft as well as glomerular capillary involvement.

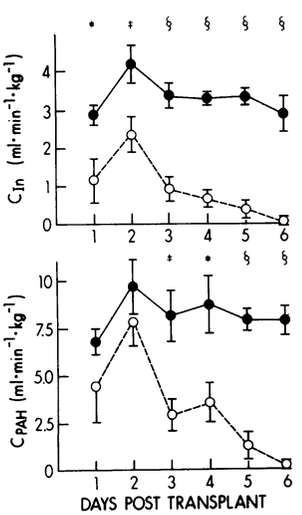

Figure 1. C_{In} and C_{PAH} of native kidneys (— ● —) and allografts (- - - o - - -) 1–6 d after transplantation ($n = 32$). Values represent means±SEM. Statistically significant differences between native and allograft values for each day are indicated by • for $P < 0.025$, ‡ for $P < 0.01$, and § for $P < 0.001$.

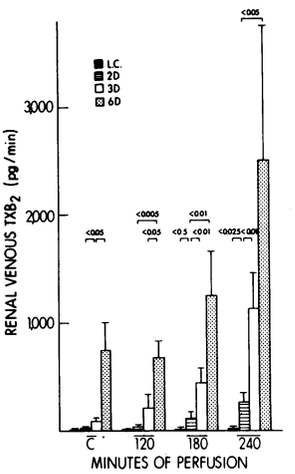

Figure 2. Renal venous TXB_2 production by ex vivo perfused Lewis control kidneys (L.C.) and 2-d (2D), 3-d (3D), and 6-d (6D) allografts. The control period (C) represents renal TXB_2 production determined for all groups before bradykinin stimulation. Renal TXB_2 production by all groups is also indicated after 120, 180, and 240 min of bradykinin stimulation (1 μg given as a bolus every 30 min). Bars represent means±SEM. Statistically significant differences between groups are indicated by brackets with the corresponding P value.

To evaluate the rejecting allograft's capacity for eicosanoid production without contamination by other tissues, we perfused allografts ex vivo with a cell-free perfusate and measured renal venous 6-keto-$PGF_{1\alpha}$, PGE_2, and TXB_2. Fig. 2 depicts renal TXB_2 production by ex vivo perfused kidneys 2, 3, and 6 d after transplantation and by nontransplanted kidneys from normal Lewis rats. Unstimulated TXB_2 production by Lewis control kidneys and 2-d allografts did not differ (28 ± 8 pg/min vs. 10 ± 8 pg/min; $n = 8$). 3-d allografts produced significantly greater amounts of TXB_2 than 2-d allografts or normal Lewis control kidneys. TXB_2 production by 6-d transplanted kidneys was significantly greater than the values of all other groups. Bradykinin stimulation produced a significant and immediate increase in TXB_2 production by all transplanted kidneys, but not by Lewis control kidneys. Unstimulated TXB_2 production by cross-clamped control kidneys and by 1-d allografts was <40 pg/min. In these kidneys, acute tubular necrosis was the predominant morphologic feature and there was no response to bradykinin stimulation.

Fig. 3 illustrates the relationship between C_{In} and unstimulated TXB_2 production in 3- to 6-d allografts. C_{In} was determined in vivo and the same kidneys were then removed and perfused ex vivo to determine unstimulated and bradykinin-stimulated TXB_2 production. There was a significant inverse linear correlation between the log of ex vivo TXB_2 production and in vivo inulin clearance ($r = 0.74$, $P < 0.05$).

The effects of daily cyclophosphamide treatment on renal allograft function and ex vivo TXB_2 production are shown in

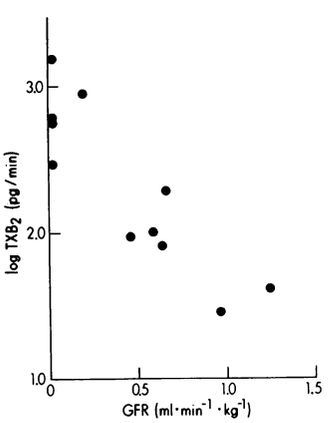

Figure 3. Relationship between unstimulated renal venous TXB_2 production and C_{In} in allografts 3–6 d after transplantation. Log of renal venous TXB_2 production before bradykinin stimulation by ex vivo perfused kidneys is plotted vs. in vivo GFR measured in the same kidneys ($r = 0.74$, $P < 0.05$).

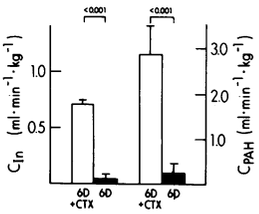

Figure 4. Effects of cyclophosphamide treatment on C_{In} and C_{PAH} 6 d after transplantation. ■, untreated animals; □, animals treated with cyclophosphamide (20 mg·kg^{-1}·day^{-1}). Values represent means±SEM. *P* values by unpaired *t* test are given.

Fig. 4 and Fig. 5. Clearances of inulin and PAH measured in cyclophosphamide-treated animals 6 d after transplantation were significantly greater than values from untreated animals (C_{In}, 0.71±0.16 vs. 0.04±0.04 ml·min^{-1}·kg^{-1}, $P < 0.001$; C_{PAH}, 2.87±0.72 vs. 0.24±0.21 ml·min^{-1}·kg^{-1}, $P < 0.001$). 6-d cyclophosphamide-treated allografts produced significantly less TXB_2 than untreated 6-d allografts both before and after bradykinin stimulation. Furthermore, cyclophosphamide treatment significantly reduced the interstitial infiltration of inflammatory cells in 6-d allografts.

Fig. 6 depicts 6-keto $PGF_{1\alpha}$ production by three groups of kidneys: (*a*) nontransplanted Lewis control kidneys; (*b*) allografts 2, 3, and 6 d after transplantation; and (*c*) 6-d cyclophosphamide-treated allografts. Ex vivo 6-keto $PGF_{1\alpha}$ production before bradykinin stimulation was similar in each group. However, after 180 and 240 min of bradykinin stimulation, 6-keto $PGF_{1\alpha}$ production by 2-, 3-, and 6-d allografts was greater than the values of either Lewis controls or the cyclophosphamide-treated rats.

As demonstrated in Fig. 7, there was no clear relationship between the number of days after transplantation and ex vivo PGE_2 production. Unstimulated PGE_2 production by 2-d allografts was significantly less than values of nontransplanted Lewis control kidneys (178±27 pg/min vs. 324±63 pg/min, $P < 0.05$) and 3-d allografts (402±118 pg/min, $P < 0.05$). After 240 min of bradykinin stimulation, PGE_2 production by both Lewis control kidneys (1,915±283 pg/min, $P < 0.005$) and 2-d allografts (1,813±456 pg/min, $P < 0.025$) was significantly greater than that of cyclophosphamide-treated rats (533±189 pg/min).

Because ex vivo TXB_2 production by allografts correlated inversely with C_{In}, we explored the functional significance of increased TX production in vivo. We acutely infused the TX synthetase inhibitor UK-37248-01 into renal arteries of seven donor kidneys 3 d after transplantation. As demonstrated in Fig. 8, in vivo RBF increased from 16.53±5.21 ml·min^{-1}·kg^{-1} before infusion to 26.19±3.45 ml·min^{-1}·kg^{-1} during infusion of UK-37248-01 ($P < 0.025$). C_{In} also increased from 1.06±0.38 ml·min^{-1}·kg^{-1} to 1.37±0.39 ml·min^{-1}·kg^{-1} ($P < 0.05$). The intra-arterial infusion of UK-37248-01 significantly reduced

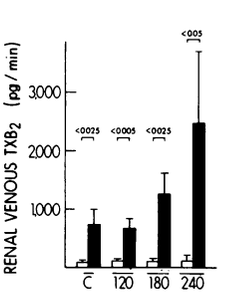

Figure 5. Effect of cyclophosphamide on renal TXB_2 production by ex vivo perfused kidneys 6 d after transplantation. ■, untreated animals; □, animals treated with cyclophosphamide (20 mg·kg^{-1}·day^{-1}). Bars represent means±SEM. *P* values by unpaired *t* test are noted.

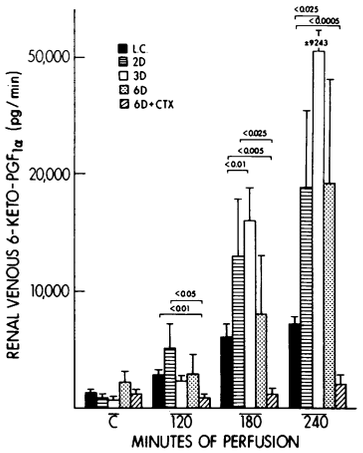

Figure 6. Renal 6-keto-$PGF_{1\alpha}$ production by ex vivo perfused Lewis control kidneys (L.C.), 2, 3, and 6-d untreated allografts, and 6-d allografts treated with cyclophosphamide. Bars represent means±SEM. *P* values are indicated where significant by unpaired *t* test.

urinary TXB_2 excretion by allografts from 1.18±0.26 pg/min to 0.50±0.22 pg/min ($P < 0.05$).

Other renal excretory data from the acute UK-37248-01 infusion studies are displayed in Table I. Mean arterial pressure was not altered by UK-37248-01 infusion (101±4 vs. 98±5 mmHg). Neither were allograft urine flow rate, percent fractional excretion of sodium, sodium excretion, potassium excretion, or percent free water clearance/osmolar clearance altered by UK-37248-01 infusion. However, as measured during the control period before UK-37248-01 infusion, native kidney C_{In}, C_{PAH}, sodium excretion, potassium excretion, and percent free water clearance/osmolar clearance were all significantly greater than values measured in the allograft. In native kidneys, acute administration of UK-37248-01 had no significant effect on any of these parameters.

Discussion

The mechanisms that reduce renal function during renal allograft rejection are unknown. Focal perivascular mononuclear cell infiltrates are a prominent morphologic feature of rejection, but the role that these cells play in altering renal hemodynamics is unclear. Various renal, endothelial, and hematopoietic cells are important sources of vasoactive hormones. In particular, both monocytes and macrophages release locally active cyclooxygenase and lipoxygenase products (16–22). These infiltrating cells may release vasoconstrictor eicos-

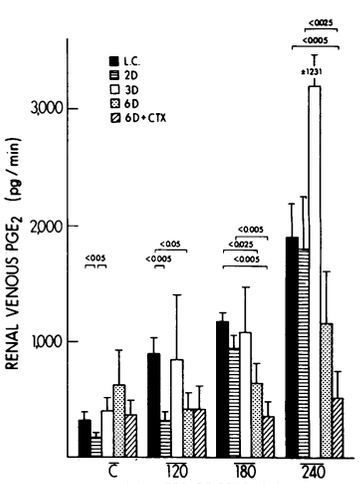

Figure 7. Renal PGE_2 production by ex vivo perfused kidneys. Experimental groups are depicted as in Fig. 6. *P* values are indicated where significant by unpaired *t* test.

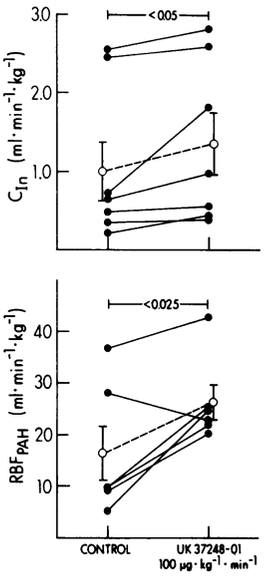

Figure 8. Effect of TX synthetase inhibition on allograft C_{In} and RBF in vivo 3 days after transplantation. RBF and C_{In} were determined before and after the renal artery administration of UK-37248-01. Individual values are connected by solid lines, mean values by dotted lines. *P* values determined by paired *t* test are noted.

anoids or stimulate renal production of vasoconstrictors, which reduce RBF and glomerular filtration rate (GFR). This hypothesis has been suggested for other models of renal disease including unilateral ureteral obstruction (11), renal vein constriction (23), and acute nephrotoxic serum nephritis (24). Because mononuclear cell infiltration is so impressive in rejecting allografts, renal transplantation rejection provides an ideal setting in which to study the effects of these cells on renal function.

In this study, we developed a model of renal transplantation rejection that was predictable in progression and severity of renal impairment. In this model, we demonstrated that TX synthetic capacity of rejecting kidneys was significantly increased. The in vivo TX synthetase inhibitor studies suggested that increased TXA_2 production may have a role in determining renal function during allograft rejection. TX synthetase inhibition had no effect on native kidney function. Therefore, improvement in allograft function probably resulted from local rather than systemic effects of the drug. The acute infusion of UK-37248-01 into allograft renal arteries increased RBF by 58% and C_{In} by 29%. However, despite this improvement in allograft function with UK-37248-01, RBF and C_{In} remained below normal. Thus, increased TX synthesis is only one of several factors that reduce renal function during acute rejection.

The cellular source of increased TX production by rejecting kidneys cannot be determined from these studies. In our model, increased TX production by allografts correlated with progressive interstitial accumulation of chronic inflammatory cells. Stimulated macrophages, lymphocytes, and leukocytes can synthesize TXA_2, PGE_2, 6-keto-$PGF_{1\alpha}$ as well as a number of lipoxygenase products (16–22). Thus, inflammatory cells that infiltrated the allograft could have been the primary source of increased eicosanoid production. In addition, lipoxygenase products released by leukocytes can stimulate TX synthesis in other cells. Feuerstein et al. (25) reported that incubation of rat peritoneal macrophages with leukotrienes C_4 and D_4 enhances the release of PGE_2, 6-keto $PGF_{1\alpha}$, and TXB_2 in a dose dependent manner. Therefore, in this study, leukotrienes from infiltrating leukocytes could have stimulated production of eicosanoids by inflammatory cells or renal parenchymal cells. Finally, platelet aggregation may also occur in the vessels of rejecting kidneys. Platelets produce large quantities of TXA_2 (26), and they too could have contributed to the increased TX production by ex vivo perfused allografts.

In other models of acute renal failure, leukocytes have been implicated as a potential source of eicosanoids or as stimulators of increased eicosanoid production. Okwegawa et al. (11) have suggested that infiltrating mononuclear cells are instrumental in the exaggerated renal TX and PG synthesis that occurs with ureteral obstruction in rabbits. Schwartz et al. (23) have demonstrated in the same species a similar phenomenon associated with renal vein constriction. Lefkowith et al. (27) have reported that treatment of hydronephrotic rabbits with nitrogen mustard decreases leukocytic infiltration and inhibits bradykinin-stimulated thromboxane production. Davis and Needleman (28) demonstrated that pretreatment with cyclophosphamide inhibits TX synthesis in hydronephrotic rabbit kidneys. In our study, cyclophosphamide treatment reduced both bradykinin-stimulated and unstimulated TXB_2 production. This inhibition of TX production was associated with a marked decrease in interstitial mononuclear cell infiltration and an improvement in both C_{In} and C_{PAH}.

Renal tissue may also be an important source of enhanced prostanoid production by rejecting kidneys. Isolated glomeruli from normal rats synthesize PGE_2, 6-keto-$PGF_{1\alpha}$, and TXB_2 (29). Isolated glomeruli produce increased amounts of TXB_2 during ureteral obstruction (30), acute nephrotoxic serum nephritis (24), and glycerol-induced acute renal failure (31). We have also shown that isolated proximal tubule suspensions from normal rats can produce PGE_2 and TXB_2 (32). Thus, altered prostanoid synthesis by the kidney, by infiltrating cells, or by an interaction between these tissues may play a role in the vasoconstriction of allograft rejection.

Acute allograft rejection has become an important clinical problem because of the frequent use of renal transplantation as a mode of therapy for end-stage renal disease. One of the major problems in evaluating nonfunctioning allografts has

Table I. Excretory Function of 3-d Transplant and Native Kidneys Before and After Administration of UK-37248-01

	C_{In}	C_{PAH}	$\dot{V}$	$\%EF_{Na}$	$U_{Na}V$	U_KV	$\%TCH_2O/C_{Osm}$
	ml/min/kg	*ml/min/kg*	*μl/min/kg*	*μeq/min/kg*	*μeq/min/kg*	*μeq/min/kg*	*μeq/min/kg*
Allograft control±SE	1.06±0.38	4.10±1.45	18.13±3.69	2.44±1.00	1.84±0.48	1.50±0.36	38.14±11.85
Allograft + UK-37248±SE	1.37±0.39*	5.21±1.54*	19.67±2.58	1.70±0.47	1.90±0.41	1.99±0.56	40.79±8.99
Native control±SE	4.16±0.50‡	10.75±1.50‡	25.86±4.04	0.72±0.14	4.21±0.97*	5.14±0.84*	81.38±0.78‡
Native + UK-37248±SE	4.35±0.35	10.94±1.30	26.86±4.09	0.81±0.11	4.68±0.70	4.84±0.48	80.48±0.98

* $P < 0.05$ vs. allograft control; ‡$P < 0.005$ vs. allograft control.

been the difficulty in differentiating acute tubular necrosis from acute cellular rejection. In this study, allografts with acute tubular necrosis 1–2 d after transplantation produced minimal amounts of TXB_2, as did cross-clamped controls. However, kidneys 3–6 d after transplantation produced significantly increased amounts of TXB_2. This observation suggests that, under these experimental conditions, increased renal TXB_2 production may differentiate acute cellular rejection from acute tubular necrosis. Furthermore, as much as 10–20% of the reduced GFR and RBF measured in 3-day allografts could be attributed directly to increased TX production. This observation may have implications for the possible use of specific TX synthetase inhibitors in the future therapy of acute rejection. In this study, cyclophosphamide profoundly reduced renal prostanoid production by the rejecting kidney. Corticosteroids, which are currently used in treating acute cellular rejection, also alter eicosanoid metabolism by inhibiting phospholipase A_2 (33). Therefore, one of the beneficial effects of corticosteroids on renal function during allograft rejection may be related to reduced TXA_2 synthesis. Since much of the morbidity of acute rejection is attributable to drug therapy, improving function with TX synthetase inhibitors may permit reduced immunosuppressive therapy.

We were unable to detect a clear relationship between rejection and PGE_2 and 6-keto $PGF_{1\alpha}$ production by ex vivo perfused allografts. There was a tendency for the ratios of PGE_2/TXB_2 and 6-keto $PGF_{1\alpha}/TXB_2$ to decrease progressively from days 2–6 after transplantation. These ratios were also lower in rejecting kidneys compared with nontransplanted, normal Lewis kidneys. The marked differences in the observed renal synthetic capacity of TXB_2, PGE_2, and 6-keto $PGF_{1\alpha}$, and the different responses to bradykinin stimulation and cytolytic therapy suggest that these eicosanoids may be produced by several different tissue types.

In summary, we developed a model of renal allograft rejection that was predictable in onset and degree of functional impairment. Renal TX synthesis in rejecting rat renal allografts was increased and this increase played a role in decreasing RBF and GFR. Although TX was not clearly the sole mediator of this decrease in renal function, its effects may be significant. The exact cellular source of increased TX production and the mechanism of its release were not addressed in this study nor did we study other important vasoconstrictors that may contribute to altered renal hemodynamics. Our data suggest that specific pharmacologic interventions that decrease TXA_2 production during acute rejection may be useful in preserving allograft function.

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