IN-DEPTH METHODS (SUPPLEMENTAL DIGITAL CONTENT)

Rat kidney transplant model, in-depth methods

Donor nephrectomy was performed via midline incision and an operating microscope was used to visualize the donor kidney. The left ureter was ligated and the suprarenal vein, renal artery and vein were separated from each other and the surrounding tissue. The distal abdominal aorta was ligated, and above that, the aorta was cannulated with a fine silastic catheter. The donor kidney was perfused in situ with 3-5 mL of cold heparinized saline solution (1%). The renal artery and vein were ligated from their junction at the aorta and vena cava, and the kidney was removed en bloc. The graft was preserved in 4°C heparinized saline solution (1%) while recipient surgery was performed. Average cold ischemia time for donor surgery was 20 to 30 minutes.

The kidney transplant recipient underwent midline laparotomy. Renal vessels were occluded close to the origin of the aorta and vena cava using individual microvascular clamps and bilateral native nephrectomy was performed at time of transplant. The donor kidney was placed in the posterior abdominal cavity of the recipient and anastomosis of donor and recipient renal arteries, vein, and ureter was performed. Operative time for recipient surgery was 45 to 60 minutes. After completion, closure of the abdominal wall and skin was performed in two layers.

Full mismatch kidney transplants were performed with Brown Norway donor to Lewis recipient. Due to the complete mismatch nature of this model, high rates of graft and animal loss are known to occur within one week due to severe T cell mediated acute rejection.¹ To prevent unacceptable graft and animal loss, recipients were given
cyclosporine (10 mg/kg/day) via intraperitoneal injection daily until harvest at seven
days following transplant. This dose of cyclosporine has been demonstrated to be
above the minimal efficacious dose and within the dose range tolerated with minimal
side effects.¹

**Donor Specific Antibody determination**

Donor (Brown Norway, RT1-A³) splenocytes were incubated with plasma from
transplant recipients, then washed, stained, and measured by flow cytometry (BD LSR II
at the UW Immunology Core) to determine DSA. Data was analyzed by FlowJo
(TreeStar, Inc.). Nonsinglets were removed and cells gated through a lymphocyte gate
and then through a CD3⁺ gate. Mean fluorescence intensity was determined for the
population of interest. Antibodies used were: anti-IgM (clone G53-238, BD Pharmingen);
anti-IgG (Affinipure F(ab')₂ fragment, Jackson Immuno); and anti-CD3 (clone 1F4,
BioLegend).

**REFERENCES**

1. Schuurman HJ, Pally C, Fringeli-Tanner M, Papageorgiou C. Comparative efficacy of
mycophenolate sodium (MPS) and mycophenolate mofetil (MMF) with and without
Figure S1. B cell deficient animals did not generate alloantibody at 7 days posttransplant. Flow crossmatch for donor specific antibody (DSA) demonstrated production of IgM (A) and IgG (B) following transplant in wild type recipients (WT) but not in B⁻/⁻ recipients. DSA levels in B⁻/⁻ remained at the level of isotype control. Mean fluorescent intensity (MFI) * P < 0.01