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# Acceleration of Chronic Rejection of Kidney Allografts from Non-Heartbeating Donors

Igor A. Laskowski, Martin Gasser, Johann Pratschke, Wayne W. Hancock, and Nicholas L. Tilney

The authors have previously shown that dysfunction of isografts correlates with the interval of cardiac arrest of the non-heartbeating donor (NHBD). In this study, the authors investigated the nonspecific effects of donor asystole on rat allografts over time. F344 kidney donors were killed, and after 45 min the left kidney was transplanted orthotopically into Lewis (LEW) recipients (group 1,  $n = 14$ ). Organs from living donors (LD) acted as controls (group 2,  $n = 10$ ). All host animals received low-dose cyclosporine (1.5 mg/day  $\times$  10). LEW recipients of kidney isografts from NHBD (group 3,  $n = 15$ ) and LD controls (group 4,  $n = 6$ ) were examined in parallel. All animals were followed for 12 weeks. Proteinuria was assessed every 4 weeks, and tissue samples examined at 2 and 12 weeks ( $n = 3$ /time point). All animals survived. Proteinuria increased progressively in all group-1 NHBD allograft and group-3 isograft recipients. From control LD groups 2 and 4, proteinuria remained at baseline. Morphologically, group-3 kidney isografts showed early acute tubular necrosis followed later by moderate interstitial fibrosis and glomerular injury. In contrast, group-1 allografts developed massive cortical atrophy, with rapidly progressive focal and segmental glomerular proliferation and sclerosis. Iso- and allograft LD kidneys remained unaffected. The immunologically independent early and late structural changes occurring in NHBD kidney isografts are dramatically accelerated in allografted organs.

## Introduction

The current major problem in the field of organ transplantation is that the supply of donors is consistently inadequate for the growing number of recipients.<sup>1</sup> As a result, alternatives to donations from brain-dead (BD) cadavers (the primary source of organs) are being investigated. The use of living-related and living-unrelated donors (LDs) has been highly successful but composes a relatively small percentage of the whole. Transplantation of organs from "marginal" or "extended" cadaver donors has been increasingly accepted. One such source are those from non-heartbeating donors (NHBDs). Interest in this donor category has been stimulated by reports of long-term graft function and survival similar to those from BD donors and by an estimated

increase in the donor pool by as much as 100%.<sup>2-4</sup> However, the primary limitations experienced by such organs include a significantly increased incidence of delayed graft function (DGF) and primary graft nonfunction (PNF).<sup>4,5</sup>

We have previously shown that both early and late functional and structural dysfunction of isografts correlate with the period of donor cardiac arrest. If this extends < 45 min, progressive morphologic changes occur in the injured kidneys, which still allow prolonged recipient survival after transplantation. However, if cardiac arrest is > 45 min, kidney isografts develop progressive end-stage changes fatal to the recipient within 6 months. We now examine the additive effects of graft allogenicity on the behavior of NHBD rat allografts over time.

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## Methods

Inbred male Lewis rats (LEW) 8 to 10 weeks of age, weighing 200 to 250 g, served as recipients of

- F344 kidney allografts from NHBDs in group 1;
- F344 kidney allografts from LDs in group 2;
- LEW kidney isografts from NHBDs in group 3;
- and LEW kidney isografts from LDs group 4 (Harlan Sprague Dawley, Indianapolis, IN).

The left donor kidney was removed and transplanted orthotopically to the recipient with end-to-end anastomosis of the renal vessels and ureter, using 10-0 Ethilon suture (Ethicon, Inc., Somerville, NJ). Organs were cooled transiently in 4 °C saline before transplantation. The mean time of ischemia was 24 min. Contralateral nephrectomy was performed on day 10. All animals were followed for 12 weeks.

### Experimental Groups

In group 1 ( $n = 14$ ), anesthetized F344 donor rats were heparinized and killed by ether overdose. Kidneys subjected to a 45-min period of donor cardiac arrest were transplanted into LEW recipients. In group 2 ( $n = 10$ ), kidneys from LD F344 engrafted into LEW animals served as controls. Animals from both groups 1 and 2 received a short course of low-dose cyclosporin A (CsA) (1.5 mg/day) for 10 days subcutaneously. Isografts were examined in parallel and consisted of LEW recipients of kidney isografts from NHBD (group 3, 45 min of cardiac arrest,  $n = 15$ ) and LD controls (group 4,  $n = 6$ ).

### Graft Function and Survival

Proteinuria (at 24 h) was assessed spectrophotometrically as a determinate of kidney function at 2 and 12 weeks ( $n = 3$ /time point). Graft survival was determined by recipient death, as the bilaterally nephrectomized animals were solely dependent upon the function of the transplanted kidney.

### Histology

Kidneys from representative animals in all groups were harvested at 2 and 12 weeks after transplantation ( $n = 3$ /time point/group). Tissue samples were fixed, sectioned, and stained with hematoxylin and

eosin (Fisher Scientific) and periodic-acid-shiff for light microscopy.

### Statistical Analysis

Survival was assessed with Kaplan-Mayer Survival Analysis. Mean proteinuria values for each group were calculated, and Mann-Whitney test for 2 independent samples was performed. Results were found to be significantly different for  $P$  values  $< 0.05$ .

## Results

### Graft Survival and Function

There was no difference in survival between the allograft and isograft recipients of NHBD and LD kidneys, around 80% of which continued to live at the end of the relatively short 12-week follow-up. Urine protein period increased progressively in all recipients of kidney allografts, although that of NHBD kidneys (group 1) was somewhat ( $P = 0.05$ ) higher at the end of the 12-week follow-up as compared with LD allografts (group 2) ( $26 \pm 25$  mg/24 h vs  $18 \pm 31$  mg/24 h at 12 weeks). Proteinuria also increased progressively in recipients of NHBD kidney isografts (group 3) by 4 weeks after transplantation versus continuing normal function in group 4 LD controls. The values had diverged further by 12 weeks ( $40 \pm 19$  mg/24 h vs  $7 \pm 2$  mg/24 h,  $P < 0.05$ ).

### Graft Histology

NHBD allografts (group 1) developed widespread tubular injury with dense interstitial fibrosis by 2 weeks. By 12 weeks, this had progressed to massive cortical atrophy, focal and segmental glomerular proliferation and sclerosis. In contrast, group 2 kidney allografts from LDs remained relatively unaffected during the first few weeks and developed minor nonspecific tubular injury and mononuclear infiltration by 12 weeks. Group 3 NHBD kidney isografts showed florid acute tubular necrosis at 24 h. By 2 weeks, there was tubular regeneration with minimal fibrosis. By 12 weeks, however, progressive tubular atrophy and glomerulosclerosis were evident, although less than the changes seen in NHBD allografts. Group 4 LD isografts were morphologically unremarkable at 12 weeks.

"MARGINAL" CADAVER DONORS

Older (>60 years) donor with diabetes or hypertension.

## Discussion

One solution to temper the shortage of organs for transplantation is the use of those from NHBDs. In particular, however, the disadvantage of using kidneys from such a source is the increased incidence of DGF and PNF after engraftment, related to the extended period of ischemic injury integral with donor asystole. In this study, we investigated the influence of prolonged (45 min) donor cardiac arrest as a risk factor for function and survival of isografted and allografted kidneys in rats. The most severe ischemic injury that a kidney can sustain occurs following complete cessation of blood flow. The non-specific inflammatory events developing following reperfusion in isologous hosts precludes any superimposed immunological activity. When transplanted into allogeneic hosts, however, the additional effects of immunity are brought into play. It seems intuitive that injury inflicted upon a kidney, retained even for relatively short intervals in a non-heartbeating donor, negatively affects its early and late behavior. Several conclusions have been drawn from the results of these studies.

- First, the severity of initial damage to a kidney from an NHBD correlates with the period of donor asystole and warm ischemia in situ.
- Second, despite some regeneration of tubular changes by 2 weeks, chronic damage progresses thereafter.
- Third, the immunological stimulus produced in the host by the presence of an allograft adds substantially to the already severe nonspecific insult.

Acute rejection is part of the continuum between the initial donor-associated inflammation sustained by the transplanted organ and the resultant expression of host-mediated immunity. In addition to activation of the cytokine/adhesion molecule cascade by the nonspecific injury, major histocompatibility complex class I and class II antigens are up-regulated, increasing the immunogenicity of the graft.<sup>6,7</sup> The influence of acute rejection as an important risk factor for late chronic changes in kidney transplants has been emphasized in several clinical series.<sup>8</sup>

Following the cumulative nonspecific and immunologically specific injuries, graft cells respond

by repair, regeneration, and proliferation. If, however, these insults extend beyond a particular threshold, more seriously damaged cells are eliminated by apoptosis or necrosis. Despite continued healing and repair, incomplete recovery may lead to progressive fibrosis. In the isografts in this study, there are some early attempts at cellular normalization; with an additional immunological insult, allografts progress inexorably toward end-stage changes of fibrosis, tubular atrophy, and glomerulosclerosis.

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"EXTENDED" CADAVER DONORS:

Marginal cadaver donors.