Early glomerular dysfunction in human renal allografts

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Background. The long-term outcome of renal allografts is characterized by a progressive deterioration of renal function and graft loss. Our aim was to determine early glomerular functional abnormalities, before they become clinically apparent.

Methods. Glomerular hemodynamics and dextran sieving were characterized in 21 well-functioning cadaveric renal allograft recipients [normal glomerular filtration rate (GFR) and albumin excretion rate (AER), who also had a kidney biopsy with normal or minimal histological changes] and in 15 uninephrectomized kidney donors. Both groups were one to three years after transplantation or uninephrectomy.

Results. The GFR and renal plasma flow (RPF) were similar in both groups (62 ± 3 vs. 63 ± 4, and 343 ± 26 vs. 334 ± 21 mL/min/1.73 m² for GFR and RPF, in cadaveric recipients vs. donors, respectively), the AER was normal in both groups, but the mean arterial pressure was higher in renal recipients (105 ± 3 vs. 94 ± 3 mm Hg in uninephrectomy controls, P < 0.05). Despite similar levels of overall glomerular function in the two groups, the dextran sieving curve was uniformly elevated in the renal allograft recipients versus uninephrectomy controls (P < 0.05 for dextrans 38 to 66 Å). Using a log-normal glomerular pore-size distribution model to analyze potential mechanisms, the elevation in the dextran sieving curve resulted from a shift in the distribution of glomerular filtering pores to a larger size (mean glomerular pore size 46 ± 2 vs. 43 ± 2 Å for uninephrectomy controls, P < 0.05), resulting in a larger fraction of filtrate volume permeating very large pores. By morphometric analysis, the thickness of the glomerular basement membrane was increased in the allograft as compared to 2-kidney biopsy controls (614 ± 33 vs. 427 ± 22 nm, respectively, P < 0.05).

Conclusions. Even in “well functioning” renal allografts there is a glomerular dysfunction characterized by increased permeability to macromolecules resulting from a shift of the glomerular pores to a larger size. These changes could be mediated by ultrastructural alterations at the glomerular capillary or by alterations in intraglomerular hemodynamics. Early allograft dysfunction may contribute to the progressive renal insufficiency of renal allografts.

Key words: dextran sieving, permselectivity, uninephrectomy, glomerular morphometry, transplantation, hemodynamics.
uninephrectomy related to trauma has been reported to be excellent in humans, with no significant increase in the prevalence of late proteinuria or renal insufficiency [18, 19], and only a slight increase in the prevalence of hypertension [20].

Long-term studies have demonstrated that at 20 years post-transplantation only about 15 to 20% of renal transplant patients survive with a functioning graft [21]. This suggests that most renal allografts, irrespective of their functional status at one year, could be at risk for late renal failure. Our present study sought to determine if there are abnormalities in glomerular hemodynamics and glomerular capillary wall function in clinically “normal” cadaveric renal allografts one to three years after transplantation by comparing them to those of single kidney individuals who had donated a kidney for transplantation one to three years earlier. Our goal was to identify early abnormalities in glomerular function, before they are manifested clinically, to provide insight into mechanisms contributing to progressive renal insufficiency in renal allografts. We also performed a morphometric analysis of renal allograft biopsies to determine if there are ultrastructural alterations associated with abnormalities in glomerular function.

**METHODS**

**Study population**

*Cadaveric renal allograft recipients.* Twenty-one individuals were identified (12 males, 9 females) who were more than one-year post-transplantation with normal renal function [defined as a GFR >40 mL/min 1.73 m² and albumin excretion rate (AER) <25 μg/min]. Their median age was 51 years (range 21 to 72) and the mean time since transplantation was two years (range 1 to 3). The immunosuppression consisted of prednisone, cyclosporine A (CsA) and either azathioprine (N = 8) or mycophenolate mofetil (N = 13). Five patients had had a rejection episode at two weeks to three months after transplantation, which responded to a pulse of methylprednisolone (4 patients) or OKT3 (1 patient). At the time of the renal function evaluation, the maintenance dose of prednisone was 10 mg/day, and the 12-hour whole blood trough cyclosporine level was 199 ± 17 ng/mL. Eighteen of the 21 patients were receiving antihypertensive drugs: dihydropyridine calcium channel blockers (7), angiotensin-converting enzyme (ACE) inhibitors (5), or both (5); one patient was taking a loop diuretic. Of the patients on ACE inhibitors, the majority (7) was on low-dose enalapril (2.5 to 5 mg/day), and three patients were on a dose of enalapril or equivalent of 7.5 to 20 mg/day. Cadaveric donor information was obtained from the Transplant Registry. The median age of the cadaveric donors was 19 years (range 13 to 49), 13 were male and 8 female, and 18 were Caucasian and 3 African American.

A kidney biopsy had been performed in all patients within four weeks of the renal function determination as part of a protocol evaluating the use of mycophenolate mofetil or a humanized anti T-cell antibody versus conventional triple immunosuppression in renal transplantation. None of the biopsies had changes of acute rejection, chronic transplant glomerulopathy or pathological features of cyclosporine toxicity. The histological findings were “mild interstitial fibrosis and tubular atrophy” in 9 cases, “mild nonspecific changes” in 8, and normal in 4 cases. There were no statistical differences in the measured physiological parameters (see below) between patients with a normal biopsy and those with mild interstitial fibrosis, and, thus, they were grouped together for analysis.

*Control population.* Sixteen kidney donors who had donated a kidney in the previous one to three years were contacted to have their glomerular function evaluated. After renal function determination, one of the donors was found to have significant proteinuria (AER of 1440 μg/min) that had not been present at the time of kidney donation. This patient’s proteinuria was subsequently determined to be related to hepatitis B contracted sometime after kidney donation, and this patient was excluded from the analysis. Thus, 15 healthy donor individuals (7 males, 8 females; 14 Caucasian, 1 Hispanic) were studied. Their median age at the time of renal function determination was 49 years (range 29 to 71), and they were not taking medications. Both patients and control subjects consented to a study that had previously been approved by the Human Investigations Committee of Emory University. Finally, control renal biopsies were obtained from kidneys of 16 cadaveric donors (9 male, 7 female) at the time of transplantation, after the establishment of the vascular anastomosis. Their median age was 39 years (range 15 to 57); 13 were Caucasian, 2 African American and 1 Hispanic. At the time of procurement, their mean serum creatinine was 1.2 ± 0.1 mg/dL (range 0.3 to 1.8). Because of ethical reasons, kidney biopsies were not obtained in the single kidney kidney donors who had volunteered for renal function evaluation.

**Glomerular function studies**

A differential solute clearance technique was used to measure glomerular function as described [22]. The morning dose of cyclosporine was held until the completion of renal function determination, to avoid acute changes in renal hemodynamics. Briefly, an intravenous catheter was inserted in each arm to infuse clearance markers and to collect blood. After a baseline blood sample, diuresis was initiated with a 10 mL/kg water load, and maintained by drinking an equivalent volume of water to that of voided urine. Intravenous loading
doses of inulin, para-aminohippuric (PAH) acid and dext-
tran 40 were administered, followed by an intravenous
infusion to maintain target levels [22]. After a 40-minute
equilibration period, four urine collections of 30 minutes
duration were obtained after spontaneous voiding, with
blood samples taken at the beginning and end of each
urine collection. GFR was calculated as the average of
the four inulin clearances, and the RPF as the average
of the clearances of PAH, adjusted for a tubular extrac-
tion of 85% [23]. The filtration fraction was calculated
as the ratio between GFR and RPF.

The concentrations of inulin and PAH were measured
as described [24]. Plasma albumin and IgG concentra-
tions were measured by a turbidometric technique using
specific antibodies, and, in urine, by radioimmunoassay
(RIA) and enzyme-linked immunosorbent assay (ELISA),
respectively, as reported [24]. Plasma oncotic pressure
was measured by membrane osmometry and used as the
arteriolar oncotic pressure [24]. Dextran in urine and
plasma was separated by size-exclusion chromatography,
using precalibrated Sephacryl HR-300 columns (Phar-
macia Fine Chemicals, Uppsala, Sweden) and measured
by the anthrone method [25]. Fractional dextran clear-
ances were calculated by dividing the clearance of each
macromolecule by that of inulin.

The rate of glomerular filtration is determined by the
relationship between ultrafiltration coefficient and net
ultrafiltration pressure according to:

$$GFR = K_f \times P_{Uf} = K_i \times [\Delta P - \Delta \pi] \quad (Eq. 1)$$

where $K_i$ is the two-kidney ultrafiltration coefficient and
$P_{Uf}$ is the net ultrafiltration pressure (the difference be-
tween the transglomerular capillary hydrostatic pressure
($\Delta P$) and mean glomerular oncotic pressure, $\Delta \pi$). In
turn,

$$K_i = S \times k \quad (Eq. 2)$$

where $S$ is the total capillary surface available for filtra-
tion and $k$ is the intrinsic hydraulic membrane conduc-
tivity.

A mathematical model for the filtration of water was
used to compute $K_i$ from values of GFR, RPF, $\Pi_A$ and
$\Delta P$ [26]. Three of these variables (GFR, RPF and $\Pi_A$)
were measured; the fourth, $\Delta P$, cannot be measured in
humans. Based on micropuncture in animal studies
[27, 28], and indirect evidence from studies of adult hu-
mans [29, 30] a physiologic range for $\Delta P$ in humans has
been estimated between 35 and 40 mm Hg. Thus, we
computed $K_i$ at a $\Delta P$ of 40 mm Hg in all patients and con-
trols. Moreover, since transplant recipients had higher
systemic mean arterial pressure values than their unin-
ephrectomized counterparts, which could be transmitted
to the glomerular capillaries, we also computed mem-
brane parameters in transplant recipients at a $\Delta P$ of 45
mm Hg.

The glomerular hemodynamic and dextran sieving
data were analyzed according to a continuous pore-size
distribution model of the glomerular capillary wall, fol-
lowing a log-normal probability distribution [31]. Ac-
cording to this model, the distribution of pore sizes is
characterized by two parameters: the mean pore radius
($u$), and the standard deviation of the distribution ($s$).
Two additional derived parameters ($r_{5\%}$ and $r_{1\%}$),
which relate to the pore size permeating 5% and 1% of
the total filtrate volume, respectively, provide a quantita-
tive estimate of the prominence of the very large pores
of the distribution [32]. The log-normal model provided
a better fit of the dextran sieving data in these non-
proteinuric individuals than the isoporous plus shunt
model, and therefore, it was used for the analysis.

**Morphometric analysis**

Tissue from patients and control biopsies was pro-
cessed for morphometric analysis. Kidney biopsy tissue
from renal allograft recipients (3 cores, on average) was
obtained percutaneously using an automated spring-
loaded biopsy needle (Bard Monopty Biopsy Instru-
ment, Covington, GA, USA). Control kidney tissue was
obtained by a wedge open technique after the establish-
ment of the vascular anastomosis of the renal allograft.
Otherwise, the tissue was processed in both patients and
controls in an identical fashion. Briefly, tissue for light
microscopic examination was fixed in 10% neutral buf-
fered formalin, dehydrated through graded alcohols,
cleared and infiltrated and embedded in paraffin. Sec-
tions approximately 3 $\mu$m thick were stained with hemato-
ylin and eosin, periodic acid-Schiff reagent and Masson
trichrome. On average, 17 glomeruli (range 7 to 44)(
$\Pi_P$ [26]) were analyzed for each patient. The average number of
glomeruli analyzed in the 16 biopsies from control sub-
jects was 32 (range 12 to 81). A computer system con-
sisting of an Olympus microscope, video camera, dedi-
cated computer, a digitizing tablet and imaging analysis
software (Bioquant; R&M Biometrics, Inc., Nashville,
TN, USA) was used to perform the measurements. At the
light microscopic level, the number of open, segment-
tally sclerosed, and occluded glomeruli were recorded.
The glomerular tuft cross-sectional area ($A_G$) was mea-
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mm Hg.
encountering sclerosed glomeruli in a random section [23]:

$$G_1 = \frac{F_1}{F_1 + (1 - F_1)(D_1/D_2)}$$  \hspace{1cm} (Eq. 4)

where $G_1$ is the true fraction of sclerosed glomeruli, and $F_1$ is the observed fraction of sclerosed glomeruli; $D_1$ and $D_2$ are the respective mean diameters of occluded and open glomeruli calculated from $V_G$.

The fraction of cortical interstitial volume was calculated superimposing a calibrated grid and performing point counting.

Tissue was prepared for electron microscopy using standard techniques. In brief, tissue was cut into 1 mm cubes, fixed in 4% buffered glutaraldehyde, post-fixed in 1% osmium tetroxide, dehydrated through graded alcohols, propylene fixed, and infiltrated and embedded in epoxy resin. Polymerized blocks were sectioned at approximately 0.5 micrometers and toluidine blue-stained sections were used to locate two open glomeruli closest to the center of the section. Ultrathin sections (~80 nm thick) were stained with lead citrate and photographed. Photomontages of two open glomeruli (×3430) were prepared. The density of the peripheral capillary filtering surface ($S_v$), the volume densities of the glomerular capillaries ($V_{V_{cap}}$) and mesangium ($V_{V_{m}}$), and the glomerular capillary length density ($L_{V_{cap}}$) and average glomerular capillary diameter were calculated by point counting using stereological techniques [23, 34]. The peripheral capillary surface area ($S$) was calculated as the product of $S_v$ and the volume of the open glomeruli ($V_G$) determined by light microscopy. Eight to ten high-powered micrographs (×13,320) of each glomerulus were used to measure glomerular basement membrane thickness and epithelial filtration slit frequency. The thickness of the peripheral capillary basement membrane (BMT) was measured as the harmonic mean, using an orthogonal interception method. On average, 162 determinations of basement membrane thickness were made per patient. The frequency of the epithelial filtration slits was calculated as the total number of slits per total length of the peripheral capillary basement membrane captured on the photomicrographs [23].

Statistical analysis

Results are expressed as mean ± standard error of the mean (SEM), unless indicated otherwise. Comparisons between the groups were made by the unpaired Student t test. Differences between groups were considered significant if $P < 0.05$.

RESULTS

Renal transplant recipients and donors who were one to three years post-transplantation or kidney donation were selected for the study. Both renal transplant recipients and uninephrectomy controls had a similar age and gender distribution (Table 1). The time after transplantation was slightly longer, on average, in the renal recipients than in uninephrectomized donors (2.4 ± 0.2 vs 1.3 ± 0.2 years, respectively, $P < 0.05$). The serum creatinine ($S_{Cr}$) values were within the normal range (1.2 ± 0.1 mg/dL) and identical in the uninephrectomy group and in the renal transplant group, despite a reduced GFR (see below). Body weight was slightly lower in cadaveric renal recipients than in uninephrectomized donors, but both groups had similar BMI (Table 1). The mean daily cyclosporine A dose in the renal allograft recipients was 4.5 ± 0.5 mg/kg, and the mean 12-hour trough whole blood cyclosporine level was 199 ± 17 ng/mL.

Despite antihypertensive medication taken by 18 of 21 renal allograft recipients, their mean arterial pressure (MAP) was significantly higher than in uninephrectomy controls (103 ± 3 vs. 94 ± 3 mm Hg, respectively, $P < 0.05$). The GFR and RPF were similar in the renal allograft recipients and in renal donors when corrected for body surface area (Table 2), or when expressed as absolute values: 67 ± 4 versus 70 ± 4 mL/min, and 376 ± 28 versus 362 ± 32 mL/min for GFR and RPF, in transplant recipients versus kidney donor controls, respectively. Consequently, the ratio between GFR and RPF (filtration fraction, FF) was identical in both groups. There were no differences in the serum albumin and IgG concentrations (data not shown), plasma oncotic pressure or the excretion rates of albumin and IgG between renal allograft recipients and uninephrectomy controls (Table 2).

Despite almost identical overall glomerular function in the two groups, there were striking differences between the groups in glomerular permeability as detected by dextran sieving analysis. As shown in Figure 1, a generalized increase in sieving curve was present in the renal allograft group, and the fractional permeability for discrete dextran molecules was statistically significant for all dextrans larger than 36 Å (38 to 66 Å, $P < 0.05$).

To calculate the intrinsic functional parameters of the glomerular capillary, we modeled the dextran sieving curve along with the glomerular hemodynamic measurements using a log-normal pore-size model of the glomerular capillary wall. Assuming that both groups had a similar transglomerular hydraulic pressure ($DP$) of 40 mm Hg [24], the mean radius of the glomerular pore size distribution ($u$) was increased in renal allograft recipients by about 3 Å, on average, as compared to uninephrectomy controls (46 ± 2 vs. 43 ± 2 Å, respectively, $P < 0.05$); the spread of the pore-size distribution ($s$) was not different between the two groups (Table 3). A shift to a larger size in the glomerular pore-size distribution (Fig. 2) resulted in an enhanced prominence of the larger pores, reflected by the increase in the computed parameters $r^*5\%$ and $r^*1\%$ in renal allografts versus
Table 1. Clinical parameters

<table>
<thead>
<tr>
<th></th>
<th>Age(^a)</th>
<th>Gender</th>
<th>Years after surgery range</th>
<th>Serum creatinine mg/dL</th>
<th>Weight kg</th>
<th>BMI kg/m(^2)</th>
<th>Previous rejection number of patients</th>
<th>HTN</th>
<th>CsA ng/mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cadaveric renal</td>
<td>51 (21–72)</td>
<td>M/F</td>
<td>2.4 ± 0.2(^b) (1–3)</td>
<td>1.2 ± 0.1</td>
<td>78 ± 4(^a)</td>
<td>28 ± 1</td>
<td>5</td>
<td>18(^b)</td>
<td>199 ± 17</td>
</tr>
<tr>
<td>allograft recipients</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Uninephrectomy</td>
<td>49 (29–71)</td>
<td>M/F</td>
<td>1.3 ± 0.2 (1–3)</td>
<td>1.2 ± 0.1</td>
<td>85 ± 5</td>
<td>29 ± 2</td>
<td>NA</td>
<td>0</td>
<td>NA</td>
</tr>
<tr>
<td>donor controls</td>
<td></td>
<td></td>
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</tbody>
</table>

Abbreviations are HTH: hypertension, defined as requirement for antihypertensive treatment; CsA, whole blood trough cyclosporine A level; NA, not applicable; BMI, body mass index.

\(^a\) Median (range)
\(^b\) \(P < 0.05\) vs. uninephrectomy controls

Table 2. Glomerular function

<table>
<thead>
<tr>
<th></th>
<th>MAP mm Hg</th>
<th>GFR mL/min/1.73 m(^2)</th>
<th>RPF FF</th>
<th>AER(^c) (\mu g/min)</th>
<th>IgG ER(^c)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cadaveric renal</td>
<td>103 ± 3(^b)</td>
<td>62 ± 3</td>
<td>343 ± 26</td>
<td>0.19 ± 0.01</td>
<td>9 (3–22)</td>
</tr>
<tr>
<td>allograft recipients</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Uninephrectomy</td>
<td>94 ± 3</td>
<td>63 ± 4</td>
<td>332 ± 22</td>
<td>0.19 ± 0.01</td>
<td>10 (1–78)</td>
</tr>
<tr>
<td>donor controls</td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
</tbody>
</table>

Abbreviations: MAP, mean arterial pressure; GFR, glomerular filtration rate; RPF, renal perfusion flow; FF, filtration fraction; AER, albumin excretion rate; IgG ER, IgG excretion rate.

\(^a\) Median (range)
\(^b\) \(P < 0.05\) vs. uninephrectomy controls

Fig. 1. Dextran sieving curve in renal transplant recipients (●) as compared to kidney donor controls (○) \((P < 0.05\) vs. controls).

Uninephrectomy controls (72 ± 1 vs. 67 ± 1, and 82 ± 1 vs. 76 ± 1 Å, respectively, \(P < 0.05\); Table 3). There were no differences in the one-kidney ultrafiltration coefficient (\(K_u\)) between the two groups. Since the renal allograft recipients had a higher systemic mean arterial pressure than the kidney donor controls that potentially could be transmitted to the glomerular capillaries, we also calculated the membrane parameters assuming that glomerular hypertension were present in renal allograft recipients (\(\Delta P\) of 45 mm Hg) [25]. The results, shown in Table 3, indicate the assumption that a difference in the prevailing intraglomerular pressures had a negligible effect on the calculated membrane parameters \(u\) and \(s\). As indicated in Table 3 and according to Equation 1, if intraglomerular hypertension occurred in the renal allografts, the ultrafiltration coefficient would be lower to achieve the measured glomerular hemodynamics (Table 2) in renal allograft recipients.

To determine whether the measured change in glomerular permeability was associated with ultrastructural alterations, we performed a renal morphometric analysis (Table 4). In renal allograft recipients one to three years after transplantation, the prevalence of glomerular sclerosis was low (3 ± 2%) and not statistically different from that present in cadaveric kidneys at the time the kidney was transplanted (8 ± 3%). Similarly, there was no difference in the volume density of the mesangium (\(V_{VM}\)) or the mesangial volume between renal allografts and biopsy controls. Notably, the glomerular volume was not different between renal allografts one to three years post-transplantation and two-kidney biopsy controls, but the capillary filtration surface density (\(S_v\)) was higher in renal allografts than in controls \((P < 0.05)\), resulting in a higher capillary filtration surface area in renal allografts than in two-kidney controls \((P < 0.05)\). This increase in
Table 3. Glomerular filtration barrier and membrane parameters

<table>
<thead>
<tr>
<th></th>
<th>Assumed ΔP</th>
<th>Ultrafiltration coefficient, K_μ</th>
<th>Mean glomerular pore size</th>
<th>Spread of distribution</th>
<th>r^5%</th>
<th>r^1%</th>
<th>(χ^2)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mm Hg</td>
<td>units</td>
<td>μ</td>
<td>s</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cadaveric renal allograft recipients</td>
<td>35</td>
<td>17.1 ± 2.1</td>
<td>46 ± 2(^a)</td>
<td>1.21 ± 0.01</td>
<td>72 ± 1(^a)</td>
<td>82 ± 1(^a)</td>
<td>0.51</td>
</tr>
<tr>
<td>Uninephrectomy donor controls</td>
<td>40</td>
<td>7.7 ± 1.2</td>
<td>46 ± 2(^a)</td>
<td>1.21 ± 0.01</td>
<td>72 ± 1(^a)</td>
<td>82 ± 1(^a)</td>
<td>0.50</td>
</tr>
<tr>
<td></td>
<td>45</td>
<td>5.4 ± 0.7(^a)</td>
<td>47 ± 2(^a)</td>
<td>1.20 ± 0.01</td>
<td>72 ± 1(^a)</td>
<td>82 ± 1(^a)</td>
<td>0.61</td>
</tr>
</tbody>
</table>

\(^a P < 0.05\) vs controls

DISCUSSION

The overall glomerular function (GFR, RPF, FF and plasma oncotic pressure) was almost identical in renal allograft recipients and single-kidney controls, indicating there were similar adaptations to the single-kidney state over a comparable period of time. However, the dextran sieving curve was strikingly different in the two groups: the fractional dextran clearances in renal allograft recipients were uniformly elevated over the entire range of molecular radii tested (28 to 66 Å), and reached statistical significance for dextrans sized 38 to 66 Å. Modeling of glomerular function indicated that the increase in permeability was due to a shift to of the distribution of glomerular pores to a larger size. The changes in glomerular dextran permeability were associated with alterations in glomerular capillary wall structure, even though there was no evidence of significant global sclerosis or mesangial expansion.

Fig. 2. Distribution of filtrate volume permeating discrete glomerular pores in renal transplant recipients (thick line) and in kidney donor controls (thin line).

Our hypothesis was that differences in intraglomerular pores in renal transplant recipients could result from an increase in the length and/or the diameter of the glomerular capillaries. The results of our morphometric analysis indicate (Table 5) that the higher glomerular filtration area results from an increase in the mean glomerular capillary diameter, and not from a change in capillary length. Other structural alterations at the glomerular capillary wall level were found (Table 4), where the glomerular membrane was thicker in renal allografts than in controls (614 ± 33 vs. 427 ± 22 nm, respectively; \(P < 0.05\)), but the number of epithelial filtration slits per peripheral capillary length was not different between the groups. Despite the normal overall glomerular function in cadaveric recipients, the fractional cortical interstitial area was also higher in renal allografts one to three years after transplantation than in control biopsies obtained at the time of transplantation (19 ± 3 vs. 12 ± 1\(^%\) in controls, \(P < 0.05\), Table 4).
brane permeability should result in increased trafficking of macromolecules and enhanced passage of large proteins. However, these early changes were not accompanied by increased albumin and IgG excretion rates in final urine (Table 2). Under conditions of relatively low filtered protein loads, the tubules could reabsorb most of the filtered proteins so that they are not excreted in final urine in excessive amounts. For instance, Tucker, Rasch and Blantz demonstrated that in rats with early diabetes and no albuminuria, high albumin excretion rates can be found in final urine when tubular albumin reabsorption is blocked by lysine [35], whereas in control rats lysine did not increase albuminuria. This suggests that early loss of glomerular permselectivity and enhanced transglomerular passage of proteins can be masked by tubular protein reabsorption. Similarly, in Pima Indians with early diabetes, Myers et al found an increase in glomerular permeability to macromolecules and dextrans with no significant increase in albumin excretion rates [36].

Are these changes predictive of late graft loss? There have been no longitudinal studies of glomerular function and dextran sieving in renal allograft recipients, so that the significance of these findings as early predictors of late graft failure cannot be fully ascertained at this time. However, in a group of patients with chronic transplant nephropathy who had renal insufficiency and proteinuria and were studied with similar techniques, Mayer et al found a generalized increase in dextran sieving curve versus healthy controls; the increase in fractional clearance was more pronounced for dextrans (>54 Å) [13]. This resulted from a shift in the glomerular pore size distribution towards larger sizes, as observed in the present study, but the change in the glomerular pore size distribution in patients with chronic transplant nephropathy was of a greater magnitude than in our population with preserved renal function. Thus, a continuum of injury to the glomerular capillary could occur in the transplanted kidney, such that initially it may not be apparent clinically, but as the injury progresses, it becomes clinically evident by proteinuria and renal insufficiency. The present study found alterations in the glomerular basement membrane similar to—but of a lesser magnitude than—those described in patients with chronic transplant glomerulopathy, namely thickening and widening of the glomerular basement membrane [37].

What could account for these findings? In contrast to uninephrectomy controls, cadaveric renal allograft recipients were taking several vasoactive drugs (ACE inhibitors, calcium channel blockers and CsA), alone or in combination, which could affect glomerular function and dextran sieving. ACE inhibitors acutely lower the filtration fraction in uninephrectomized healthy individuals [38], but this should lower rather than increase the dextran sieving curve [31]. Moreover, in renal allograft recipients with proteinuria, ACE inhibitors cause a trend to a lower filtration fraction and a generalized depression of the dextran sieving curve [39], again, the opposite effect of what we observed. The main renal hemodynamic effect of the dihydropyridine calcium channel blockers is to increase renal blood flow [40], an effect that should lower rather than increase the dextran sieving curve [31].

Cyclosporine A acutely reduces renal blood flow by constricting the afferent arteriole, lowers GFR and in-

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**Table 4. Renal morphometric analysis**

<table>
<thead>
<tr>
<th>Renal allograft recipients</th>
<th>Vg/10^6 μm^3</th>
<th>GS %</th>
<th>Sv</th>
<th>Glomerular filtration area Sg/10^4 μm^2</th>
<th>Vvm</th>
<th>Mesangial volume Vm/10^4 μm^3</th>
<th>BMT μm</th>
<th>FSF slits/mm</th>
<th>PCL</th>
<th>Interstitial area %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Biopsy controls</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(2-kidney)</td>
<td>2.7 ± 0.2</td>
<td>3 ± 2</td>
<td>0.19 ± 0.03a</td>
<td>510 ± 74a</td>
<td>0.14 ± 0.01</td>
<td>399 ± 50</td>
<td>614 ± 33a</td>
<td>1339 ± 48</td>
<td>19 ± 3a</td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations are: Vg, volume of open glomeruli; GS, global sclerosis; Sv, glomerular capillary filtering density; Vvm, volume density of mesangium; BMT, glomerular basement membrane thickness; FSF, filtration slit frequency; PCL, peripheral capillary length.

*P < 0.05 vs controls

**Table 5. Morphometric analysis of glomerular capillaries**

<table>
<thead>
<tr>
<th>Renal allograft recipients</th>
<th>Capillary length density (LV)</th>
<th>Capillary length/glomerulus μ ×10^3</th>
<th>Average capillary diameter μm</th>
<th>Capillary volume density</th>
<th>Capillary volume/glomerulus μ^3 ×10^6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Biopsy controls</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(2-kidney)</td>
<td>0.0075 ± 0.001</td>
<td>21.1 ± 1.7</td>
<td>18.2 ± 0.6a</td>
<td>0.473 ± 0.013a</td>
<td>1.34 ± 0.12a</td>
</tr>
</tbody>
</table>

*P < 0.05 vs. controls
creases the filtration fraction [41]. These effects could reduce intraglomerular pressure, and because of its differential effect on convective and diffusive fluxes, raise the dextran sieving curve [42]. We think that a reduction in ΔP is unlikely to occur in renal transplant recipients for the following reasons: (1) renal blood flow, GFR and filtration fraction were identical in the two groups, arguing against an acute hemodynamic effect; (2) most patients were receiving dihydropyridine calcium channel blockers, agents that have been shown to counteract the acute hemodynamic effects of cyclosporine [43]; and (3) a reduction in ΔP would have to be associated with a increase in Kt to maintain GFR (Eq. 1). For instance, calculating a 5 mm Hg reduction in ΔP in transplant recipients (from 40 to 35 mm Hg) would require a doubling of the glomerular ultrafiltration coefficient versus single kidney controls to maintain GFR (Table 3), a change unlikely to be present only in the renal allografts recipients. Finally, we performed a sensitivity analysis to determine whether changes in ΔP could reproduce the observed sieving curve in renal transplant recipients, assuming that the latter group had the same membrane parameters (u and s) as the kidney donor controls. The results (data not shown) indicate that changes in ΔP without changes in the glomerular pore size distribution cannot explain the observed dextran sieving curve. A reduction in ΔP to 35 mm Hg would elevate the fractional clearance of 28 to 34 Å size dextrans (a region of the sieving curve that is not statistically different from donor controls, Fig. 1), but it would have no effect on the fractional clearance of dextrans in the 38 to 66 Å range, the region of the curve which is significantly different from controls. Taken together, we conclude that neither cyclosporine A nor other vasoactive medications taken by the renal transplant recipients could account for the observed increase in fractional dextran clearance.

A generalized increase in the dextran sieving curve has been induced experimentally in humans by infusing angiotensin II at doses that cause a slight (4 mm Hg) increase in systemic mean arterial pressure. There also is a 1 to 2 Å increase in the size of the main glomerular pores. However, in contrast to our study, angiotensin infusion markedly reduces renal blood flow and increases the filtration fraction [44]. In the rat, angiotensin II can enhance dextran permeability, which could be mediated by “stretch”-induced changes in membrane porosity, probably mediated by increases in the glomerular hydraulic pressure and ΔP [45]. It is worth noting that transplant recipients had a higher systemic mean arterial pressure than their uninephrectomy controls. This could be transmitted to the glomerular capillaries, elevate the intraglomerular hydraulic pressure and change the pore size distribution to a larger size by stretch-mediated mechanisms. It is worth noting that in uninephrectomized adult rats, glomerular capillary number, length and diameter increases in the remnant kidney by 26, 12 and 14%, respectively [46]. Unfortunately, no such studies have been performed in humans. Therefore, it is possible that renal allografts could have relative intraglomerular hypertension versus uninephrectomized kidney donors and a shift in the glomerular pore size distribution to a larger size.

The glomerular ultrastructural alterations that follow the removal of 50% of kidney mass have not been well characterized in humans. In an autopsy study, Narkum-Burgess et al did not find a significant increase in glomerular volume in World War II veterans with a solitary kidney after uninephrectomy [19]. Glomerular hypertrophy also was not present in renal allografts with preserved function as compared to two-kidney controls [47]. Unfortunately, lack of tissue preservation in these post-mortem specimens precluded a more detail analysis of glomerular substructures in those studies. In keeping with these observations, we did not find glomerular hypertrophy in well-functioning renal allografts as compared to biopsies from two-kidney controls (Table 4). On the other hand, the density of the glomerular capillary filtering surface (Sv) was increased in renal transplant recipients as compared to two-kidney controls, resulting in a larger total one-kidney capillary filtering area. This finding suggests that the renal allografts had undergone structural adaptations to the single kidney state. Also, the increase in capillary filtering area in renal allografts resulted from an increase in the mean diameter of the glomerular capillaries, rather than increases in capillary length. Interestingly, the glomerular adaptive changes were restricted to the capillary compartment, since there were no differences in the mesangial density or total mesangial area between the two groups (Table 4). However, at the glomerular capillary wall level (the structural barrier to macromolecular filtration), renal allografts had a thickened glomerular basement membrane, with no change in the number of epithelial filtration slits per unit of capillary length. This early increase in glomerular basement membrane thickness may be important, because we have found that the thickness of the glomerular basement membrane is increased further in patients with chronic transplant nephropathy who had renal insufficiency (unpublished observations), suggesting that early structural alterations may be involved in the mechanisms of progressive renal insufficiency in renal allografts. For example, a thickened glomerular basement membrane could increase the resistance to water flow across the glomerular capillaries and lower the hydraulic glomerular membrane conductivity. Early increases in basement wall thickening could be offset by increases in the total glomerular filtering area, so that Kt, the product of the hydraulic membrane conductivity and total filtering area, would be unchanged. Unfortunately, there are no published studies of glomerular capillary ultrastructure in
long-term survivors after uninephrectomy with preserved renal function to determine whether the structural adaptations to the single kidney state differ between renal allograft recipients and uninephrectomized individuals. We emphasize that the control morphometric values were obtained at the time of transplantation from individuals with two kidneys, and these kidneys had not undergone the histological changes that accompany the adaptations to the single kidney state. As mentioned in the Methods section, ethical reasons precluded obtaining kidney biopsies from healthy kidney donors. To our knowledge, a detailed glomerular morphometric analysis of a solitary kidney after uninephrectomy has not been reported in healthy humans.

In summary, this study demonstrates differences in the functional adaptation to a single kidney state between cadaveric renal allograft recipients and uninephrectomized individuals, despite almost identical overall glomerular function. There is an increase in glomerular permeability to macromolecules and glomerular pore size even in well-functioning cadaveric renal allograft recipients one to three years after transplantation. Structurally, there were alterations in the glomerular capillary wall. We postulate that early alterations in glomerular permeability, associated with increased transglomerular protein trafficking (and possibly increased intraglomerular pressure) may contribute to the development of late sclerosis and graft failure.

ACKNOWLEDGMENTS

This study was supported in part by PHS grant (M01-RR00039) from the General Clinical Research Centers Programs and by the Baxter Extramural Grant Program. Our study was presented in part at the 30th Annual Meeting of the American Society of Nephrology, San Antonio, November 2-5, 1997.

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APPENDIX

Abbreviations used in this article are: ACE, angiotensin-converting enzyme; AER, albumin excretion rate; A, glomerular tuft cross-sectional area; BMT, basement membrane; CsA, cyclosporine A; ΔP, transglomerular hydraulic pressure; Δπ, glomerular oncotic pressure; FF, filtration fraction; GFR, glomerular filtration rate; Kf, ultrafiltration coefficient; LV, glomerular capillary length density; MAP, mean arterial pressure; PAH, paraaminohippuric acid; Psl, net ultrafiltration pressure; RPF, renal plasma flow; S, surface area; s, standard deviation of the distribution; Sv, glomerular capillary filtering surface; u, mean pore radius; Vg, glomerular volume; Vus, volume density of the glomerular capillaries; Vus, volume density of the mesangium.

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