NDT

Nephrology Dialysis Transplantation

Original Article

Nephron number determines susceptibility to renal mass reduction-induced CKD in Lewis and Fisher 344 rats: implications for development of experimentally induced chronic allograft nephropathy

Attila J. Szabo[1], Veronika Muller[2], Gin-Fu Chen[3], Lennie J. Samsell[4], Aaron Erdely[5] and Chris Baylis[3]

[1]Department of Pediatrics, [2]Department of Pulmonology, Semmelweis University, Budapest, Hungary, [3]Department of Physiology and Functional Genomics and Department of Medicine, University of Florida, Gainesville, FL, [4]Department of Pediatrics, West Virginia University and [5]Toxicology and Molecular Biology Branch, National Institute of Occupational Safety and Health, Morgantown, West Virginia, USA

Abstract

Background. The Fisher 344 (F344) rat kidney transplanted to a Lewis rat recipient is a common model of chronic renal allograft nephropathy (CAN); however, CAN does not develop when the Lewis kidney is grafted into a F344 recipient. In this study we investigated whether a difference in nephron number/glomerular volume exists between the strains that could contribute to a greater susceptibility to development of kidney disease in the F344.

Methods. Separate animals, male F344 and Lewis rats, were subjected to either sham surgery or right uni-nephrectomy and infarction of 2/3 of the left kidney, to produce a 5/6 ablation/infarction injury (5/6 A/I). Serial urinary protein excretions were measured, a terminal 24-h creatinine clearance was obtained and rats were killed 11 weeks after surgery and kidneys were harvested for pathology. Glomerular volumes were measured in the sham controls of each strain. Glomerular number was counted in separate, normal rats of each strain.

Results. The normal F344 had ∼30% fewer glomeruli than Lewis rats that were larger in volume. The sham F344 had similar creatinine clearance and glomerular structure to the Lewis shams, although BP and urine protein excretion were higher. After 5/6 A/I the F344 developed more severe proteinuria and structural kidney damage. When factored for kidney weight, the F344 rats exhibited a greater compensatory hyperfiltration in response to 5/6 A/I, compared to Lewis.

Conclusions. The F344 strain is more vulnerable to development of progressive kidney damage due to 5/6 A/I, compared to the Lewis. This is likely due to the lower nephron number/greater glomerular volume of the F344 that may also account for the greater susceptibility to CAN exhibited by this strain.

Introduction

Nephron number endowed during fetal development determines later susceptibility to hypertension and chronic kidney disease (CKD), and genetic background is a major determinant of nephron number [1–4]. When few functional glomeruli are present they tend to hypertrophy, thus increasing volume, and increased glomerular volume predicts later development of hypertension and progressive renal disease [5,6]. Thus, glomerular number is likely to influence the development of any specific CKD.

The most widely used experimental model of chronic renal allograft rejection is the Fisher 344 (F344) kidney to the Lewis rat recipient transplantation model [7,8]. This represents a relatively mild immunological 'mismatch', and if acute rejection is suppressed with short-term immunosuppression, the chronic rejection and subsequent chronic allograft nephropathy (CAN) develop slowly over several months. Interestingly, when the Lewis rat kidney is grafted into the F344 recipient the kidney transplants *do not* develop CAN [8]. This highlights the importance of alloantigen-independent factors in the development of CAN.

There are alloantigen-independent donor-related factors that can impact on late allograft function, which can also influence the rate of progression of other forms of chronic kidney disease (CKD), including nephron volume and number [1–3,9–12]. In this study we investigated (1) the glomerular number/volume in the normal kidney of the two strains and (2) the relative vulnerability of F344 and Lewis rats to an alloantigen-independent model of CKD, produced by right uni-nephrectomy and infarction of 2/3 of the left kidney (5/6 A/I).

Correspondence and offprint requests to: Chris Baylis, Department of Physiology and Functional Genomics and Department of Medicine, 1600 SW Archer Road, Room M554, University of Florida, PO Box 100274, Gainesville, FL 36210, USA. Tel: +1-352-392-7869; Fax: +1-352-392-7935; E-mail: baylisc@ufl.edu

Table 1. Summary of body weight (BW) and kidney weight (KW) in the F344 and Lewis sham (S) and 5/6 A/I (A/I) rats

	BW, g baseline	BW, g 11 weeks	KW, g right	KW, g left	KW/BW
F344 S	208 ± 2	329 ± 6	1.17 ± 0.07	1.12 ± 0.04	0.0036 ± 0.0002^a
F344 A/I	201 ± 6	297 ± 10	0.72 ± 0.02	1.46 ± 0.08	0.0036 ± 0.0002^b
P value	ns	ns	<0.001	<0.01	ns
Lewis S	$240^* \pm 2$	$351^* \pm 6$	$1.45^* \pm 0.06$	$1.24^* \pm 0.04$	0.0041 ± 0.0002
Lewis A/I	$240^* \pm 2$	$348^* \pm 7$	$1.02^* \pm 0.02$	$1.85^* \pm 0.07$	$0.0043^* \pm 0.0003$
P value	ns	ns	<0.001	<0.001	ns

*Significant difference from respective Fisher 344 group, $P < 0.05$.
aFor shams the right kidney harvested at sacrifice is compared to the sacrifice BW.
bFor 5/6 A/I rats the right kidney harvested at the time of surgery is compared to the baseline BW.

Subjects and methods

Inbred male F344 (RT1v1, from Harlan Sprague-Dawley, Indianapolis, $n = 19$) and male Lewis (RT1, from Harlan Sprague-Dawley, Indianapolis, $n = 17$) were purchased at 10–12 weeks of age. All animals were maintained on standard rat chow and water *ad libitum*. Sham surgery and 5/6 ablation/infarction (A/I) were performed on rats of each strain as follows: the A/I surgery involved lateral flank incision to remove the right kidney and ligate branches of the left renal artery to infarct ∼2/3 of the left kidney mass. A/I and sham surgery (without removal of renal mass) were done under general anesthesia (using a 1:1 mixture of the barbiturate anesthetics methohexital and pentobarbital, each given at 2.5 mg/100 gBW, ip). The surgery was conducted under full sterile conditions and with the approval of the West Virginia University Animal Care and Use Committee. Twenty-four-hour urine collections were made in control (pre-surgery), and at Weeks 2, 4, 6, 8, 9, 10 and 11 after surgery in the studies to assess total protein excretion and creatinine excretion at Week 11. Total protein was determined using the Bradford assay and creatinine was measured by HPLC using an assay described by us previously [1]. Blood pressure (BP) was measured under anaesthesia via aorta just prior to killing; then blood was collected and tissues were perfused with PBS and harvested. Plasma creatinine was determined as above and BUN was measured using a Sigma kit (640-A).

In subsequent studies glomerular counting was conducted on five normal rats of each strain, aged ∼14 weeks using the method of Damadian *et al.* [13]. Briefly, the left kidney was decapsulated, medulla removed and discarded and cortex weighed, and then cut into six pieces and incubated in 15 ml of 5 N HCl at 37°C for ∼80 min. The HCl was removed, the kidney washed and then incubated in 50 ml of H_2O for ∼24 h at 4°C. Samples were then brought to room temperature, gently macerated with a pestle and brought up to exactly 150 ml with H_2O. An even suspension was created by gently swirling the mixture, and 2–3 × 0.5 ml aliquots were placed in 35 mm² culture dishes bearing a 3.5-mm grid and total glomeruli were counted under 40× power.

Histology was performed on kidneys from A/I and sham rats fixed in 10% formalin and then embedded in paraffin wax. Sections of 5 µm were cut and stained (PAS) and examined, blinded, for the frequency and level of glomerulosclerosis. Injury was assessed using a 0–4+ scale, where 1+ = up to 5%, 2+ = 26 = 50%, 3+ = 51–75% and 4+ = 76% – global glomerular sclerosis. The overall glomerular injury scale was calculated as (0 × no. of +0) + (1 × no. of +1) + (2× no. of +2) + (3 × no. of +3) + (4 × no. of +4) × 100/no. of glomeruli measured. The mean glomerular volume (V_g) was also calculated for the sham rats of both strains, from the planar cross sectional area using the formula V_g = mean glomerular area$^{3/2}$ × 1.38/1.1 of 30–45 undamaged cortical glomeruli/kidney.

Statistics were obtained by the unpaired *t*-test, Wilcoxon rank-sum analysis, repeated measure ANOVA and least-squares means, and data are given as mean ± SE. Statistical significance is assumed when $P < 0.05$.

Results

At ∼12–14 weeks of age, in the baseline state the body weight (BW) and kidney weight (KW) of the F344 rats were significantly less than those of the Lewis rats while the KW/BW ratio was similar (Table 1). Over the 11-week observation period both rat strains showed increases in BW and KW in the sham control group, with the F344 BW increase being greater than that in the Lewis, $58 \pm 3\%$ versus $46 \pm 2\%$ ($P < 0.05$). In the sham controls (at 11 weeks after sham surgery), Pcr was higher in F344 ($P < 0.005$) although BUN and Ccr (total and factored for kidney weight) were not different between the F344 versus Lewis rats (Table 2). The mean BP was higher in the F344 versus Lewis rats ($P < 0.001$). There is no difference in the percentage of sclerosed glomeruli in the young adult sham rats of each strain (Table 2; and the overall glomerular injury scale was similar in Lewis and F344 shams, 2.2 ± 0.4, range 1–4 versus 4.0 ± 0.7, range 2–6; ns), although the $U_{prot}V$ was greater in F344 versus Lewis shams (16 ± 1 versus 8 ± 1 mg/24 h, $P < 0.005$). The individual glomerular volume, measured in the shams, was significantly higher in the F344 versus the Lewis rats (1.56 ± 0.05 versus 1.31 ± 0.03 mm³ × 10^{-3}, $P < 0.001$). In the separate groups of age-matched normal rats in which the total number of glomeruli/kidneys were counted, we observed that the F344 had significantly fewer glomeruli than the Lewis rat ($27\,131 \pm 1668$ versus $34\,512 \pm 1549$, $P < 0.01$, $n = 5$ for each).

After 5/6 renal mass reduction (A/I) both strains exhibited similar increases in BW with time to their respective shams ($48 \pm 4\%$ versus $45 \pm 3\%$) while the functioning remnant 1/6 kidney (scar tissue removed) showed

Table 2. Indices of kidney function, structure (percentage of glomeruli sclerosed, GS) and blood pressure (BP) in the F344 and Lewis sham and 5/6 A/I rats

	Pcr (mg/dl)	BUN (mg/dl)	Ccr (ml/min)	Ccr (ml/min/gKW)	BP (mmHg)	GS (%)
F344 S	0.59 ± 0.04	17 ± 1	4.1 ± 0.6	1.85 ± 0.29	103 ± 2	4 ± 1
F344 A/I	1.07 ± 0.13	47 ± 5	2.4 ± 0.3	1.71 ± 0.30	107 ± 5	56 ± 3
P value	<0.01	<0.001	<0.05	ns	ns	<0.001
Lewis S	0.41* ± 0.01	21 ± 2	5.4 ± 0.4	2.04 ± 0.19	75* ± 3	2 ± 1
Lewis A/I	0.68* ± 0.03	32* ± 2	3.2 ± 0.3	1.37 ± 0.41	84* ± 4	29* ± 2
P value	<0.001	<0.005	<0.005	0.05	ns	<0.001

*Significant difference from respective Fisher 344 group, *P* < 0.05.
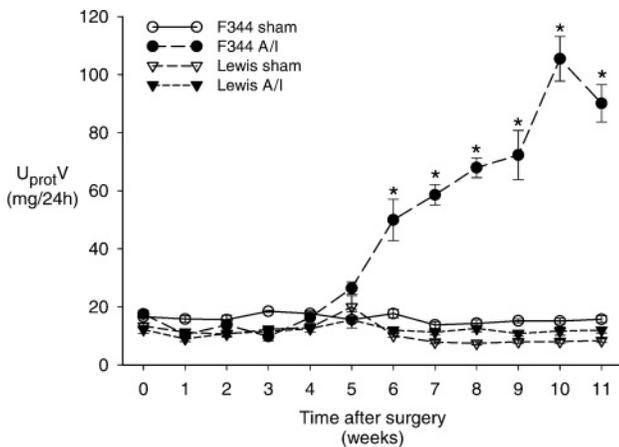
Fig. 1. The 24-h urinary total protein excretion ($U_{prot}V$) in the baseline state (Week 0) and following either 5/6 renal mass reduction by ablation/infarction (A/I) or sham surgery, in F344 and Lewis, male rats. *denotes a significant (*P* < 0.05) change from baseline.

significant and similar hypertrophy in F344 (103 ± 9%) and Lewis (81 ± 9%) rats 11 weeks after surgery (Table 1).

In response to 5/6 A/I, the F344 rats developed significant proteinuria, first being evident ~6 weeks after reduction of renal mass (Figure 1). In contrast, the 5/6 A/I Lewis rats had no change in urine protein excretion over the 11-week period. BUN and Pcr increased after 11 weeks of 5/6 A/I in both strains, but to greater absolute values (Table 2) in the F344. The total GFR (estimated from 24 h Ccr) was lower in the A/I rats of each strain versus respective shams. When Ccr was expressed in terms of functional kidney weight, there was no change after A/I in F344 but in Lewis this value was reduced after A/I. There were no strain differences in the Ccr/gKW in either shams or A/I. The arterial BP did not change with 5/6 A/I in either F344 or Lewis rats although the absolute BP was significantly lower in Lewis than in F344 (A/I *P* < 0.005). The total percentage of sclerosed glomeruli was higher in the F344 versus Lewis rats 11 weeks post-5/6 A/I (Table 2). We also assessed the severity of injury/glomerulus that was more extensive in F344 versus Lewis post-A/I, with the F344 A/I showing >20% of glomeruli with global (4+) damage compared to <5% in the Lewis A/I rats. There was a marked increased in overall injury in the Lewis A/I rat versus Lewis sham (66.8 ± 7.8, range 48–96, *P* < 0.005) and the injury seen in the F344 with A/I was even greater (*P* < 0.002) than in the Lewis A/I (166 ± 9.1, range 120–192).

Discussion

The main finding in this study is that the F344 rat develops more severe CKD in response to 5/6 A/I than the Lewis rat, and that this is associated with fewer nephrons and greater glomerular volume in the vulnerable, F344 strain. Since the F344 and Lewis rats are commonly used to produce an experimental model of CAN, this 'mismatch' in nephron number between donor (F344) and the Lewis recipient is likely to contribute to the development of CAN.

There are many alloantigen-independent donor-related factors that can influence the long-term survival of a renal transplant [14], and nephron number seems to be a major determinant. Brenner and Milford originally hypothesized that when nephron under-dosing occurs in a renal transplant, i.e. where nephron supply is inadequate for the recipient demand, this contributes importantly to development of CAN [15]. Experimental support was obtained in the F344 to Lewis allograft model where graft survival correlated directly with transplanted nephron number, and in the presence of two, rather than a single donor kidney, CAN was virtually absent [10,16]. Also, the appearance of CAN with a single transplanted kidney was delayed when the second native kidney was retained for 8 weeks post-transplant, and only developed after removal of the native kidney [10]. In the present study we report that the F344 has ~30% fewer glomeruli with greater volume than the Lewis rat, and since the F344 is the usual donor, this is likely to accelerate the development of CAN in this allograft model. Indeed, the nephron excess in the Lewis is the likely explanation why CAN does not develop when a Lewis donor kidney is grafted into a F344 recipient [8].

Nephron number also influences the rate of progression of other forms of CKD [1–3]. In this study we observed that the nephron-deficient F344 exhibited much more rapid progression of CKD compared to Lewis rats when subjected to an alloantigen-independent model of CKD, produced by right uni-nephrectomy and infarction of 2/3 of the left kidney. There were marked compensatory increases in GFR (estimated from 24 h Ccr) in the remnant in both strains but the Ccr factored for viable kidney remnant remained unchanged in F344 while falling in the Lewis. This, together with the fewer number of nephrons in the F344, implies proportionally greater compensatory increases in SNGFR in F344 than Lewis in the remnant. There were no measurements of glomerular haemodynamics in the present study, but it is likely that the greater the compensatory hyperfiltration response to nephron loss, the more marked the

afferent arteriolar vasodilation and consequent glomerular hypertension are, a primary risk factor for progression of CKD [4].

As shown here, a lower nephron number correlates with greater volume of the individual glomeruli. In the present study the total glomerular filtration area (nephron number × individual glomerular volume) was approximately equal in F344 and Lewis despite the lower nephron number and higher glomerular volume in the F344 (42.3 and 45.2 mm^3 respectively). The original hypothesis by Brenner, Garcia and Anderson [4] suggested that there was an inverse relationship between the total glomerular filtration surface area and risk of hypertension and progression. However, increased glomerular volume is a risk factor for progression of CKD [17], and it now appears that many small glomeruli are preferable to fewer larger glomeruli and that the larger the individual glomerular volume, the greater is the tendency to develop hypertension and progressive renal disease [5,6]. The mechanism by which increased glomerular volume causes renal damage is likely due to increases in intramural tension as glomerular volume (diameter) increases [17]. Thus glomerular hypertrophy and glomerular hypertension act synchronously to exacerbate development of CKD.

There are many factors that determine nephron number, some of which are likely to be genetically mediated. There are several examples of inbred rat strains that exhibit a low nephron number and susceptibility to CKD, such as F344 (present study) and the Munich Wistar Frompter [18]. Conversely, Wistar Furth rats have a high nephron number and are resistant to several forms of CKD compared to Sprague-Dawley [1,2]. In man, there is clear evidence that a low nephron number associates with hypertension and CKD. There is an astounding almost 10-fold variation in the nephron number in man of various ethnic origins, but there is no clear evidence of any genetic association. What is clear is that early developmental events program the kidney for nephron number and that a hostile uterine environment leading to fetal malnutrition is associated with a reduced nephron number and a later susceptibility to hypertension and CKD [19,20].

In summary, this study provides another example of an association between an inborn nephron deficit and susceptibility to later development of CKD. This study has particular relevance to the experimental renal transplant literature since the donor kidney widely used in the rodent model of CAN is derived from the F344 rat, which has a low nephron number. We recognize the complexity of the processes leading to CAN but consider that the nephron number makes a significant contribution to the non-immunological causes of CAN.

References

1. Erdely A, Wagner L, Muller V *et al*. Protection of Wistar Furth rat from chronic renal disease is associated with maintained renal nitric oxide synthase. *J Am Soc Nephrol* 2003; 14: 2526–2533
2. Erdely A, Freshour G, Smith C *et al*. Protection against puromycin aminonucleoside (PAN) induced chronic renal disease in the Wistar Furth. *Am J Physiol (Renal)* 2004; 287: F81–F89
3. Fogo A. Animal models of FSGS: lessons for pathogenesis and treatment. *Semin Nephrol* 2003; 23: 161–171
4. Brenner BM, Garcia DL, Anderson S. Glomeruli and blood pressure. Less of one, more the other? *Am J Hypertension* 1988; 1: 335–347
5. Keller G, Zimmer G, Mall G *et al*. Nephron number in patients with primary hypertension. *New Eng J Med* 2003; 348: 101–108
6. Schmidt K, Pesce C, Liu Q *et al*. Large glomerular size in Pima Indians: lack of change with diabetic nephropathy. *J Am Soc Nephrol* 1992; 3: 229–235
7. White E, Hildemann WH, Mullen Y. Chronic kidney allograft reactions in rats. *Transplantation* 1969; 8: 602–617
8. De Heer E, Davidoff A, van der Wal A *et al*. Chronic renal allograft rejection in the rat. Transplantation-induced antibodies against basement membrane antigens. *Lab Invest* 1994; 70: 494–502
9. Li M, Nicholls KM, Becker GJ. Risk factors for late renal allograft dysfunction: effects of baseline glomerular size. *J Nephrol* 2002; 15: 620–625
10. Azuma H, Nadeau K, Mackenzie HS *et al*. Nephron mass modulates the hemodynamic, cellular, and molecular response of the rat renal allograft. *Transplantation* 1997; 63: 519–528
11. Abdi R, Slaky D, Kittur D. Baseline glomerular size as a predictor of function in human renal transplantation. *Transplantation* 1998; 66: 329–333
12. Erdely A, Greenfeld Z, Wagner L *et al*. Sexual dimorphism in the aging kidney: inverse relationship between injury and nitric oxide system. *Kidney Int* 2003; 63: 1021–1026
13. Damadian RV, Shwayri E, Bricker NS. On the existence of non-urine forming nephrons in the diseased kidney of the dog. *J Lab Clin Med* 1965; 65: 26–39
14. Nankivell BJ, Chapman JR. Chronic allograft nephropathy: current concepts and future directions. *Transplantation* 2006; 81: 643–654
15. Brenner BM, Milford EL. Nephron underdosing: a programmed cause of chronic renal allograft failure. *Am J Kidney Dis* 1993; 21: 66–72
16. Mackenzie HS, Tullius SG, Heemann UW *et al*. Nephron supply is a major determinant of long-term renal allograft outcome in rats. *J Clin Invest* 1994; 94: 2148
17. Daniels BS, Hostetter TH. Adverse effects of growth in the glomerular microcirculation. *Am J Physiol* 1990; 258: F1409–F1416
18. Fassi A, Sangalli F, Maffi R *et al*. Progressive glomerular injury in the MWF rat is predicted by inborn nephron deficit. *J Am Soc Nephrol* 1998; 9: 1399–1406
19. Hoy WE, Hughson MD, Bertram JF *et al*. Nephron number, hypertension, renal disease and renal failure. *J Am Soc Nephrol* 2005; 16: 2557–2564
20. Schreuder MF, Nauta J. Prenatal programming of nephron number and blood pressure. *Kidney Int* 2007; 72: 265–268