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Development of injury in a rat model of chronic renal allograft rejection: effect of dietary protein restriction

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Abstract Non-allogeneic factors such as increased nephron "workload" may contribute to chronic renal allograft rejection. Reducing dietary protein from 20% to 8% was tested in a model of chronic rejection: Dark Agouti kidney to Albino Surgery recipient, "tolerised" by previous donor blood transfusions. Survival, weight gain, serum creatinine concentration and creatinine clearance were similar for both groups at all times. Urinary protein was significantly ($P < 0.05$) lower in the low-protein (LP) group 1 month after transplantation. After 3 and 6 months, both groups demonstrated mild chronic rejection. After 6 months, tubular atrophy was significantly ($P < 0.05$) less in the LP group and interstitial fibrosis was marginally reduced. Glomerular hypertrophy, glomerular sclerosis, tubular dilatation, leucocyte infiltration, adhesion molecule expression and TGF-β_1 mRNA expression were similarly increased in both groups. Thus, reducing dietary protein to 8% lowered urinary protein, but did not significantly affect the development of chronic rejection in renal allografts beyond affording a degree of protection from tubulointerstitial damage.

Key words Renal transplantation, chronic rejection, dietary protein restriction · Dietary protein restriction, chronic rejection, renal transplantation · Chronic rejection, dietary protein restriction, renal transplantation

Introduction

Renal transplantation has become a successful treatment for patients with end-stage renal failure. Advances in the control of acute rejection have led to the steady improvement of short-term graft and patient survival rates. Despite this, the survival of long-term grafts remains unchanged, with approximately 50% of grafts being lost by 10 years after transplantation [24, 26, 27]. This irreversible process is known as chronic rejection and is defined as the progressive, functional deterioration of transplanted tissue occurring months or years after engraftment [2].

Clinically, chronic renal allograft rejection is characterised by a continual decline in renal function, proteinuria and hypertension. Histopathologically, chronic rejection encompasses low-grade cellular infiltration, fibrosis, tubular atrophy, glomerulosclerosis and fibro-obliterative vascular disease [24, 35]. The mechanisms involved in chronic rejection are as yet not fully elucidated, but both allogeneic and non-allogeneic factors are thought to contribute to the development of injury [2, 6, 24, 27, 35].

Experimental and clinical studies in chronic renal disease have shown that lower dietary protein can ameliorate chronic renal damage [4, 7, 19, 21, 27, 33, 34]. Experimentally, dietary protein restriction has been shown to ameliorate renal injury in the remnant kidney model [21, 33] and in puromycin aminonucleoside nephritis [7, 19]. Clinically, the "Modification of Diet in Renal Disease Study" demonstrated a benefit of protein restriction in advanced renal disease, but failed to do so

conclusively in cases of moderate renal disease [9, 14, 18].

The suggested mechanisms responsible for the beneficial effects of dietary protein restriction are multiple and include reduction of glomerular capillary pressure, decrease of renin synthesis, reduction of ammoniagenesis and other metabolic actions [13, 19, 30, 31, 34]. The general premise of dietary protein restriction is its ability to reduce the nephron "workload", potentially reducing the development of chronic renal injury.

Transplantation of a single kidney represents a 50% reduction in renal mass, and further loss of nephrons can occur through a degree of trauma, ischaemia and reperfusion injury, and the immune response. It is suggested that the increased "workload" of the remaining nephrons contributes to the development of chronic injury [3, 8, 15, 40]. Thus, dietary protein restriction could have potentially beneficial effects. To date, there have been relatively few clinical studies, only on small numbers of renal transplant recipients, and these demonstrated decreased proteinuria and plasma renin activity in patients with chronic renal allograft rejection receiving low-protein diets [13, 30, 31].

To further examine the potential benefit of dietary protein restriction in reducing chronic renal allograft damage, this study investigates the effect of a low-protein diet in a rat model of chronic renal allograft rejection, developed in our laboratory [11, 35], and analyses the immunopathological consequences of this dietary intervention.

Materials and methods

Rats

Male, adult (3–6 months) inbred Dark Agouti (DA) and Albino Surgery (AS) rats were obtained from Monash Animal Services. All procedures were performed under ether anaesthesia. All rats received care in compliance with the guidelines of the National Health and Medical Research Council, and with the approval of the Monash Medical Centre Animal Experimentation Ethics Committee B and the Monash University Standing Committee on Ethics in Animal Experimentation.

Diets

All rats were maintained on the standard diet until they received a kidney transplant. Kidney recipients were then randomly assigned to each diet and were kept on that diet throughout the follow-up.

Standard diet (ND)

Mouse breeder diet (Barastoc Stockfeeds Pty, Pakenham, Victoria, Australia) was the standard diet. This pelleted diet consists of: protein at least 20%, fat 6.6%, carbohydrates (unspecified) at least 65%, fibre 3%, methionine 0.3% and choline 0.25%.

Low-protein diet (LP)

The pelleted diet was prepared by CSIRO, Division of Human Nutrition (O'Halloran Hill, South Australia, Australia), according to the American Institute of Nutrition AIN89A formulation for a semi-purified diet for rats and mice with an 8% protein content. The diet was refrigerated and was used within 3 months of manufacture. The components were: casein 8%, corn starch 59.2%, sucrose 12%, corn oil 10%, cellulose 5%, DL-methionine 0.3%, choline bitartrate 0.2%, mineral mix (ICN Biomedicals) 3.5% and vitamin mix (ICN Biomedicals) 1%.

Chemical analysis confirmed that protein content was 8.1%. The major fatty acid components were linoleic, oleic, palmitic and stearic acids (fatty acid profile: 16:0 = 10.9, 18:0 = 3.1, 20:0 = 0.5, 22:0 = 0.4; 18:1 = 28.7, 20:1 = 0.4, 18:2 = 55.2, 18:3 = 0.8%). This diet contains more phosphorus than the ND (1.7% vs 0.7%) because casein has a higher phosphorus content than the animal protein in ND. Other mineral, vitamin and amino acid content was similar to ND.

Transplant model

As previously described [10, 11], all AS recipients received 1 ml of heparinised DA blood, 10 and 6 days before the kidney allograft. Cyclosporin A (5 mg/kg per day) was administered by gavage for 8 days until 4 days before transplantation. Untreated DA kidneys were transplanted orthotopically into the treated AS recipients. The recipient's left renal artery was "telescoped" into the donor renal artery and the left renal vein was reconstituted using an external cuff on the donor vein. Donor and recipient ureters were joined over a short stent. Both recipient kidneys were removed at the time of transplant. Rats received no further immunosuppressive therapy and were placed on either the ND ($n = 29$) or the LP ($n = 37$) diet.

Follow-up

Blood was collected for the estimation of serum creatinine and urea concentration. Fortnightly, rats were weighed and placed in metabolic cages for 24-h urine collection. Creatinine clearance and proteinuria were estimated. Rats were killed at 1 month (ND = 5, LP = 6), 3 months (ND = 5, LP = 6) and 6 months (ND = 4, LP = 5) after transplantation. Kidneys were removed, weighed and bisected longitudinally. One-half was fixed in 10% neutral formalin for histology. One-quarter was snap-frozen in liquid nitrogen for mRNA analysis. The remaining quarter was embedded in OCT for immunohistochemistry.

Control group

Data from the transplant recipients was compared to that from a group of DA and AS normal rat kidneys (NRK; $n = 8$ histology; $n = 4$ immunohistochemistry; $n = 6$ Northern blots).

Histology

Sections of formalin-fixed, paraffin-embedded tissues were stained with haematoxylin and eosin, periodic acid-Schiff or silver Masson trichrome and assessed by light microscopy. The severity of the following changes was graded from 0 (normal) to 4 (severe): glomerular sclerosis, tubular dilatation, tubular atrophy and interstitial fi-

brosis. Glomerular hypertrophy was assessed using image analysis (Image Pro Plus 3.0; Media Cybernetics, Maryland, USA). The area of ten glomerular sections cut through the vascular pole was measured for each sample, with results expressed as the mean ± SEM area of glomeruli (μm^2).

Immunohistochemistry

Monoclonal antibodies (mAbs) against several cell surface markers were used, namely, OX1 (anti-rat CD45), ED1 (anti-rat monocytes and macrophages), R73 (anti-rat α/β T cell receptor), 3.2.3 (anti-rat NK cells), ICAM-1 (anti-intracellular cell adhesion molecule-1), LFA-1 (anti-leucocyte function antigen-1), α-SMA (anti-α-smooth muscle actin), TGF-β (anti-human transforming growth factor-β, cross reactive with the rat) and bFGF (anti-bovine basic fibroblast growth factor, cross reactive with rats and humans). OX1 was obtained as a cell line from the European Centre for Animal and Cell Culture, (Salisbury, England, UK). ED1 was a gift from Dr. C. Djikstra (the Netherlands). 3.2.3 was a gift from Dr. R. de Bruin (the Netherlands). R73, ICAM-1, LFA-1 and TGF-β were from Serotec, (Oxford, England, UK). α-SMA was from Sigma-Aldrich, (St. Louis, Mo., USA). bFGF was obtained from UBI, (Lake Placid, NY, USA).

Cryostat sections (8 μm) of kidneys snap-frozen in OCT were stained using a 4-layer immunoperoxidase technique [37]. Briefly, sections were post-fixed in 4% paraformaldehyde and non-specific staining was blocked by preincubation with 10% normal rabbit serum and 10% foetal calf serum in PBS with 0.01% sodium azide before overnight incubation with the primary antibody at 4°C. A second layer, goat anti-mouse IgG (Sigma), was applied for 30 min at room temperature. Endogenous peroxidase activity was blocked and the third layer, rabbit anti-goat immunoglobulin (Dako), and fourth layer, goat peroxidase anti-peroxidase (Dako), were applied, each for 30 min at room temperature. The reaction was developed by the addition of metal-enhanced diaminobenzidine substrate (Pierce Rockford, Ill., USA), and slides were counterstained in Harris haematoxylin.

Infiltrating leucocytes were quantitated by counting positively labelled cells in each section using a 100-square graticule at a magnification of 400 ×. Seven adjacent graticules were counted, each corresponding to an area of 0.15 mm^2. The immunostaining for adhesion molecules (ICAM-1 and LFA-1) and α-SMA was assessed based on the pattern and area of distribution of each protein in the section. They were graded as N (equivalent to that in NRK) or from 1 to 4 (increased expression detected in < 25%, 26%–50%, 51%–75% or > 75% of the section, respectively).

Northern blot analysis

Total RNA was extracted from approximately 0.2–0.6 g kidney tissue snap-frozen at nephrectomy, using guanidine isothiocyanate, and purified by cesium chloride gradient centrifugation [5]. Northern blot analysis for quantification of TGF-β_1 mRNA was performed as previously described [36]. Briefly, the RNA was denatured by treatment with dimethylsulfoxide/glyoxal, electrophoresed in a 1% agarose horizontal gel in phosphate buffer and blotted onto nylon membrane (Hybond-N, Amersham). A 370-bp TGF-β_1 cDNA probe, labelled with ^{32}P-dCTP using random primers (Megaprime, Amersham, Buchinghamshire, UK), was used. Blots were prehybridised at 60°C for 4–16 h with buffer containing 0.1–0.2 mg/ml herring sperm DNA (Sigma), 50% deionised formamide, 5 × SSPE buffer, 5 × Denhardt's reagent, 0.1% SDS, and hybridised overnight at 60°C in the same buffer containing radiolabelled probe (10^6 cpm/ml). Non-specifically bound probe was removed by washing in 2 × SSC / 0.1% SDS at room temperature for 20 min, followed by 25 min in 0.2 × SSC / 0.1% SDS at 60°C. Quantitative variability of either isolation, transfer or loading of RNA was controlled for by reprobing the blots with a 720-bp cDNA probe for GAPDH, at a hybridization temperature of 42°C. Blots were exposed for 7–72 h to a phosphor imager plate, and the bands of TGF-β_1 and GAPDH mRNA were measured using a Fuji Bio Imager Analyzer. Results are expressed as the ratios of TGF-β_1/GAPDH. Six different NRK standards were run on every blot to enable comparisons between blots. The mean TGF-β_1/GAPDH ratio for the six NRK controls was standarised to a value of 1.

Rat TGF-β_1 and GAPDH cDNA probes

Rat TGF-β_1 and GAPDH cDNA probes were synthetised by RT-PCR of rat kidney RNA using primers based on the published sequences [28, 39]. The PCR products were subcloned into pGEM-4z (GAPDH) or pGEM-3z (TGFβ_1) plasmids and validated by sequencing.

Statistics

Results were analysed by the Kruskal-Wallis 1-way ANOVA and the Mann-Whitney *U*-test using SPSS for Windows.

Results

Survival and renal function

Rat survival was essentially similar in both the ND and LP groups (Fig. 1). Thirteen recipients failed to develop tolerance and grafts were lost to acute rejection during the first 3 weeks after transplantation. A further 8 rats from the ND group and 13 from the LP group died prematurely, with a median survival time of 44 (range 28–89) and 47 (range 22–92) days, respectively. Serum creatinine (SCr) concentrations at their last follow-up point were 330 ± 71 and 455 ± 72 μmol/l, respectively (median ± range). Thus, renal failure was demonstrated in all but 3 rats (1 ND, 2 LP) that had SCr values below 100 μmol/l.

All recipients lost weight in the first 2 weeks after transplantation and regained weight thereafter. The rate of weight gain was similar for both ND and LP diets.

Both ND and LP groups demonstrated one or two episodes of rejection during the first 3 weeks after transplantation as shown by sharp peaks in SCr (Fig. 2). After the 3rd week, a steady improvement in renal function was evident, although SCr in both groups remained consistently elevated above the normal range of 41–65 μmol/l for the duration of the experiment. At no time point was there a significant difference in SCr between ND and LP (Fig. 2). Values for creatinine clearance (CrCl) were within the normal range and similar in both groups at all time points (Table 1).

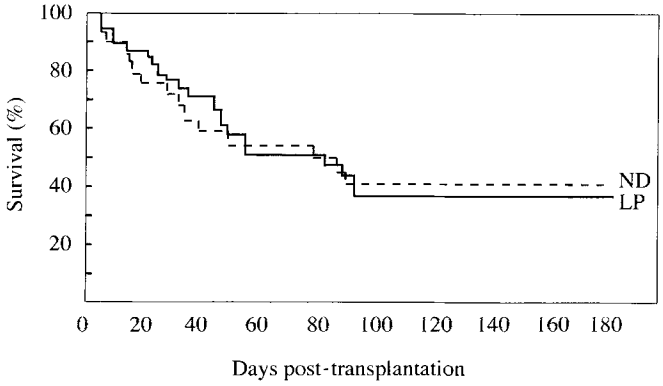

Fig. 1 Survival of kidney allograft recipients on ND and LP diets

Table 1 Renal function parameters in ND and LP rats (mean ± SEM)

Diet and month	Creatinine clearance (ml/min) (normal range 0.7–1.9 DA rat; 0.5–1.6 AS rat)	Protein output (mg/24 h) (normal range 18–22 DA rat; 0–108 AS rat)
ND 1 month	0.6 ± 0.09	30 ± 7
LP 1 month	0.4 ± 0.08	14 ± 3*
ND 3 months	0.7 ± 0.2	42 ± 11
LP 3 months	0.7 ± 0.09	17 ± 3
ND 6 months	0.9 ± 0.07	49 ± 20
LP 6 months	0.8 ± 0.2	29 ± 11

* $P < 0.05$ vs 1M ND protein output

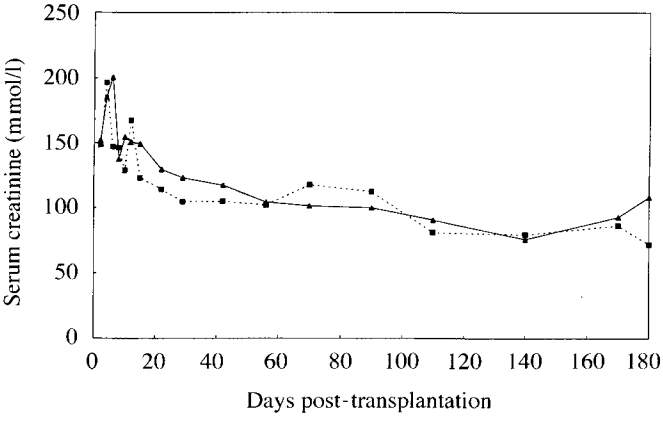

Fig. 2 Serum creatinine values for allograft recipients on ND (-■-) and LP (—▲—) diets

Urinary protein output was significantly ($P < 0.05$) higher in ND than in LP rats 1 month after transplantation. Protein output in ND rats was outside the normal range for DA animals, but remained within the normal range for AS rats (Table 1).

Histopathology

The main histopathological characteristics of the LP and ND rats are described in Table 2. At 3 and 6 months after transplantation, all of the rats in the ND and LP groups exhibited changes that were indicative of mild or moderate chronic rejection. There were focal areas of damage and considerable variability between individuals (Fig. 3a–d).

The average glomerular areas for normal DA and AS rats were 6049 ± 1121 and 6842 ± 537 μm^2, respectively. Significant glomerular hypertrophy (Table 2) was evident from 1 month after transplantation, consistent with the effect of reduced renal mass. A further increase in glomerular size with time was evident in both groups, with glomeruli being significantly enlarged at 3 and 6 months compared to 1 month in the LP group, and at 6 months compared to 1 month in the ND group ($P < 0.05$). Comparison of glomerular sizes between the LP and ND groups at the different times showed no significant differences.

Glomerular sclerosis and vascular changes were mild in all kidneys examined. Mild to moderate tubular dilatation was evident from 1 month after transplantation and was a feature of all grafts. Tubular atrophy increased with time, but was more prominent in the ND group (Fig. 3d) and was significantly more severe in ND than in LP rats 6 months after transplantation. A similar trend was observed for interstitial fibrosis ($P = 0.0591$).

Immunohistochemical analysis

The number of cells infiltrating the grafts was maximal at 1 month, exhibiting a diffuse and focal distribution, with the latter around vascular and glomerular structures. After 3 and 6 months, the infiltrate was reduced and exhibited a more prominent focal distribution. Similarly, the adhesion molecules ICAM-1 and LFA-1 were upregulated in the chronic rejection (CR) model, ICAM-1 being expressed on endothelium, tubular structures and infiltrating leucocytes, and LFA-1 being prominent on infiltrating cells. The low-protein diet did not result in a decrease in inflammatory cell infiltration, or adhesion molecule expression: the degree of cellular infiltration, ICAM-1 and LFA-1 expression was similar in the ND and LP groups at all time points (Table 3). α-SMA is normally expressed by vascular smooth muscle cells. After injury it is a marker of the phenotypic change from fibroblasts to myofibroblasts and can also be expressed by activated mesangial cells. Both groups of rats demonstrated similar consistent upregulation of α-SMA in non-vascular structures of the interstitium which did not decline significantly over the course of

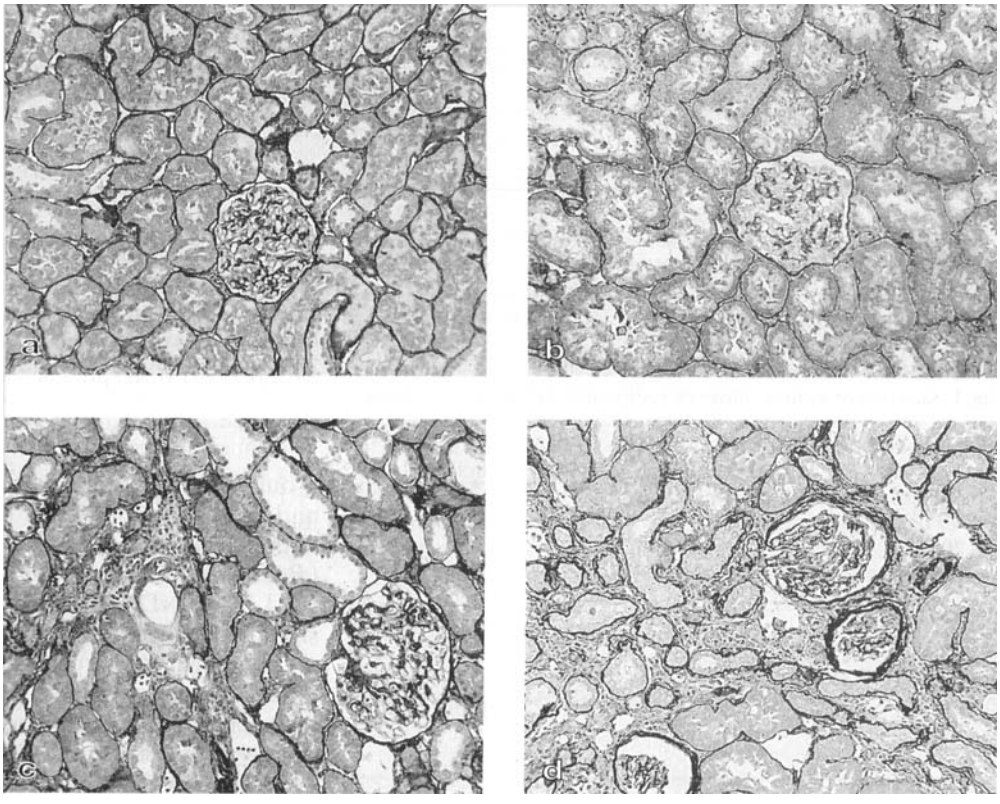

Fig. 3a–d Variability of damage between allograft recipients on ND and LP diets (silver Masson trichome, × 168): **a** LP rat at 6 months post-transplantation demonstrating mild damage; **b** ND rat at 6 months post-transplantation demonstrating mild damage; **c** LP rat at 6 months post-transplantation demonstrating moderate damage with cellular infiltration, tubular dilatation and mild glomerular sclerosis; **d** ND rat at 6 months post-transplantation demonstrating moderate damage with cellular infiltration, interstitial fibrosis and tubular atrophy

the experiment (Table 3). The α-SMA-positive interstitial myofibroblasts were localised predominantly to perivascular, peritubular and periglomerular sites with damage (Fig. 4b). Thus, the LP diet did not appear to have a significant effect on myofibroblast activation.

Growth factor expression

TGF-β_1 mRNA levels were upregulated in both groups throughout the study, being maximal at 1 month (Table 4). Thus, the dietary protein restriction did not have a direct effect on TGF-β_1 expression. TGF-β immunoreactivity was mainly demonstrated in infiltrating cells, and the degree of staining appeared maximal at 1 month, parallelling the degree of infiltration by macrophages of the grafts.

Basic FGF immunoreactivity was also similar in both groups of rats, being prominent in intertubular cells in the medulla and restricted to vascular smooth muscle cells and occasional interstitial cells in areas of damage in the cortex. Occasional mesangial cells were also positive (Fig. 5a, b). There was no difference in immunoreactive bFGF between ND and LP rats groups at any time point.

Table 2 Histopathological characteristics of ND and LP grafts (mean ± SEM)

Group	Glomerular hypertrophy (μm²)	Glomerular sclerosis	Vascular damage	Tubular dilatation	Tubular atrophy	Interstitial fibrosis
ND 1 month	7385 ± 905*[1]	0.4 ± 0.2	0 ± 0	2.0 ± 0.3	0.4 ± 0.4	0.6 ± 0.2
LP 1 month	6749 ± 353*[1]	0.3 ± 0.3	0.2 ± 0.2	1.8 ± 0.4	0 ± 0	0 ± 0
ND 3 months	9186 ± 661*[1,*2]	0.7 ± 0.5	0.7 ± 0.7	2.0 ± 0	1.8 ± 0.7	1.3 ± 0.6
LP 3 months	8600 ± 703*[1]	0.5 ± 0.4	0.3 ± 0.2	1.8 ± 0.3	0.7 ± 0.3	0.6 ± 0.2
ND 6 months	11002 ± 1003*[1,*2]	0.5 ± 0.2	0 ± 0	1.3 ± 0.3	1.1 ± 0.3	1.2 ± 0.3
LP 6 months	9836 ± 834*[3]	0.5 ± 0.2	0 ± 0	1.3 ± 0.3	0.5 ± 0.5*[4]	0.7 ± 0.3*[5]

*[1] $P < 0.05$ vs normal DA and AS; *[2] $P < 0.05$ vs 1M ND;
*[3] $P < 0.05$ vs 1M LP; *[4] $P < 0.05$ vs 6M ND; *[5] $P < 0.06$ vs 6M ND

Table 3 Immunohistochemical analysis of ND and LP grafts (*N* normal expression)

	NRK	1 month		3 months		6 months	
	$n = 4$	ND ($n = 5$)	LP ($n = 6$)	ND ($n = 5$)	LP ($n = 6$)	ND ($n = 4$)	LP ($n = 5$)
OX1	153 ± 19[a]	608 ± 128*	496 ± 68*	380 ± 130*	430 ± 40*	269 ± 97*	325 ± 34*
R73	42 ± 14	333 ± 99*	340 ± 57*	206 ± 42*,**	321 ± 61*,**	155 ± 14*,**	191 ± 27*,**
ED1	145 ± 18	228 ± 30*	247 ± 26*	190 ± 44*,**	234 ± 40*,**	86 ± 19*,**	138 ± 34*,**
3.2.3	21 ± 5	138 ± 34*	145 ± 8*	78 ± 10*,**	131 ± 48*,**	46 ± 13*,**	49 ± 3*,**
ICAM-1	N	3.4 ± 0.3[b]	3.2 ± 0.6	1.7 ± 0.3	2.3 ± 0.3	1.5 ± 0.3	2.0 ± 0.4
LFA-1	N	3.0 ± 0.5	3.1 ± 0.4	1.7 ± 0.3	2.2 ± 0.3	1.7 ± 0.2	1.7 ± 0.2
α-SMA	N	2.2 ± 0.2	2.1 ± 0.2	1.5 ± 0.3	1.1 ± 0.2	1.5 ± 0.4	1.6 ± 0.4

[a] Positive cells/mm^2 (mean ± SEM)
[b] Expression of ICAM-1, LFA-1 and α-SMA was graded as described in Material and Methods (mean ± SEM)
* $P < 0.05$ vs NRK; ** $P < 0.05$ vs 1 month

Table 4 TGFβ_1 expression in ND and LP grafts

	NRK	1 month		3 months		6 months	
	($n = 6$)	ND ($n = 5$)	LP ($n = 3$)	ND ($n = 4$)	LP ($n = 4$)	ND ($n = 3$)	LP ($n = 4$)
TGF-β_1/GAPDH	1 ± 0.04[a]	3.8 ± 0.5*	3.8 ± 0.4*	1.9 ± 0.3*,**	2.2 ± 0.4*,**	2.3 ± 0.2*,**	2.5 ± 0.4*,**

[a] Northern blot analysis was performed as described in Materials and Methods. Results are expressed as the ratio of TGFβ_1/GAPDH (mean ± SEM), with normal values standardised to a mean value of 1
* $P < 0.05$ vs NRK; ** $P < 0.05$ vs 1 month

Discussion

This study has examined the effect of reducing protein intake from 20% to 8% in a rat model of chronic rejection. A minor protective effect on tubular structures was demonstrated, but no observed improvement in renal function or survival was apparent, despite decreased urinary protein output in the LP group. Survival, serum creatinine concentrations, creatinine clearance, glomerular changes, cellular infiltration and growth factor expression were similar in both groups. Overall results were consistent with previous data demonstrating that the tolerisation regimen was successful in approximately 75% of recipients in this strongly rejecting DA-to-AS combination [11, 35].

This model of chronic renal allograft rejection in the rat, tolerised by donor-specific blood transfusions prior to transplantation, may not have involved sufficient damage for the benefit of protein restriction to become apparent, since creatinine clearances were within the normal ranges in both groups of rats. This is consistent with results obtained in clinical renal disease by the "Modification of Diet in Renal Disease Study" [18], which examined the effect of dietary protein restriction and strict blood pressure control in patients suffering from chronic renal failure. A beneficial effect of protein restriction was demonstrated in patients with advanced renal disease whilst in patients with moderate renal disease the correlation between protein intake and rate of decline in GFR was not conclusive, although further

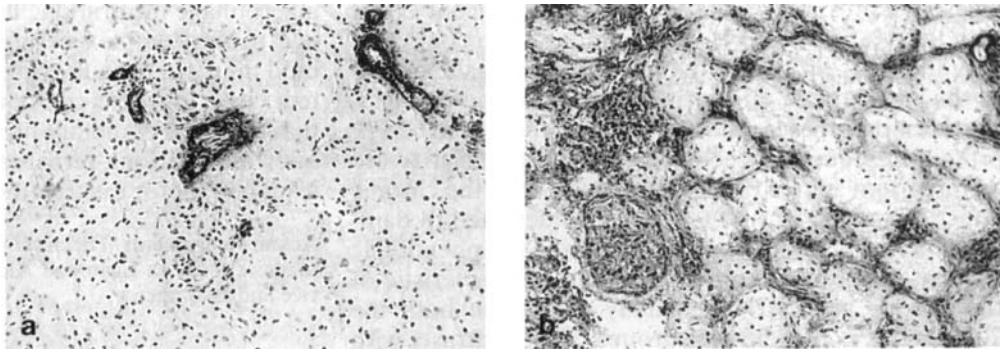

Fig. 4 a, b Expression of α-SMA (× 168): **a** expression of α-SMA in NRK is localised to vascular smooth muscle cells of arterioles; **b** LP rat at 6 months post-transplantation demonstrating perivascular, periglomerular and peritubular expression of α-SMA

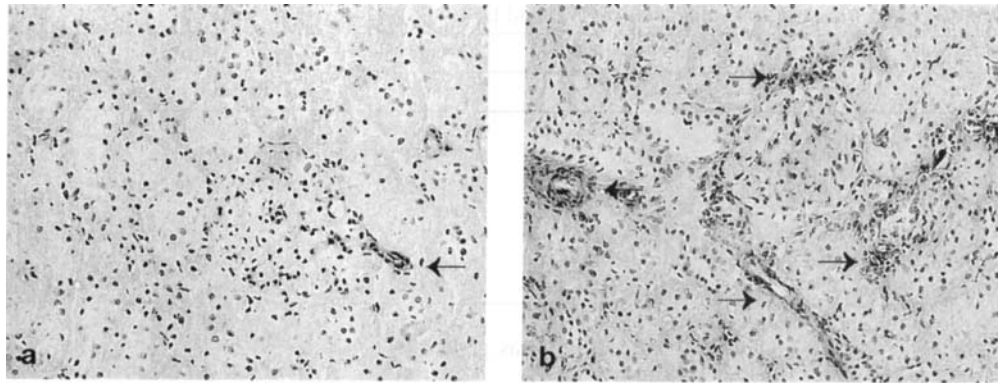

Fig. 5a, b Expression of bFGF (× 168): **a** expression of bFGF in NRK cortex is restricted to vascular smooth muscle cells; **b** LP rat at 6 months post-transplantation demonstrating perivascular and interstitial expression of bFGF

analysis suggested a beneficial effect of protein restriction [9, 14, 18].

Glomerular hypertrophy was prevalent in both the LP and ND groups, suggesting the possible need for greater protein restriction, despite significantly reduced total urinary protein outputs in the LP group. Reducing protein intake to 5% or 6%, in place of 8%, may have reduced hypertrophy but would also have had an adverse effect on overall growth and would have lowered serum protein. The results in the rat in this study were similar to those in a 3 -year clinical trial of protein restriction on the progression of renal disease in children, where the protein level had been limited to one that would not restrict growth: there was no significant effect on the decrease in renal function [43].

Histological analysis of grafts demonstrated a degree of protection from tubulointerstitial damage in the LP group. It has been suggested that, in renal disease, a high-protein diet can increase O_2 consumption in the tubules, resulting in increased ammoniagenesis and increased generation of reactive O_2 species [20, 22]. Also, abnormal reabsorption of proteins can induce tubulo-interstitial damage through functional alterations in the tubules, resulting in upregulation of vasoactive and inflammatory mediators.

It may not be valid to assume that similar mechanisms contribute to the development of renal damage in different renal diseases with a variable aetiology. In the present study, all groups of rats demonstrated increased expression of TGFβ_1, but no significant effect with the 8% protein diet was apparent when compared with the 20% protein diet. No reduction was apparent in the numbers of infiltrating monocyte/macrophages. Furthermore, they appeared to co-localise with TGFβ_1 protein in sequential sections, suggesting that they are an important source of this growth factor in the chronic rejection model. Basic FGF was also detected in damaged areas from both groups of rats, and the phenotypic change of fibroblast to myofibroblast, characteristic of the fibrotic process, was equally evident in both the 20% and 8% protein diet groups. This contrasts with findings in experimental glomerulosclerosis and nephrotic syndrome, where researchers have shown that dietary protein restriction will reduce PDGF and TGFβ expression [7]. However, no such effect on TGFβ_1 expression could be demonstrated in the remnant kidney after 5/6th nephrectomy, although significantly lower infiltrating monocyte/macrophages were reported [33].

The process of chronic renal allograft rejection is complex, and it is thought to involve both allogeneic and non-allogeneic mechanisms of injury. There is evidence in long-surviving allografts of a continuing allogeneic response, albeit dampened, when compared to the acute rejection response. This is demonstrated by the persistence of graft-reactive cytotoxic and helper T splenocytes and donor reactive alloantibodies in the recipient's circulation after long-term engraftment [17, 23, 29, 38, 42]. Non- allogeneic factors include ischaemia and reperfusion injury [40, 41, 44, 45], reduced functioning renal mass [8, 15, 16], hypertension [12, 25, 26, 32] and hyperlipidaemia [1]. The chronic rejection process possibly reflects the dysregulation of the inflammatory/tissue repair mechanism elicited in response to injury, involving broad cytokine networks and resulting in cell proliferation and excess matrix deposition. Based on the effect of dietary protein restriction in renal disease, it was expected that the 8% protein diet would have been of benefit by reducing the impact of loss of functioning renal mass and by possibly attenuating some of the fibrotic changes that derive from growth factor overexpression and monocyte/macrophage infiltration. This was not the case, possibly due to the complex nature of the injury, the relatively mild injury in the surviving animals and, to a certain extent, its allogeneic component. These findings suggest that a degree of dietary protein restriction that does not interfere with long-term growth is unlikely to be of significant benefit for the treatment of chronic rejection.

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