Progression to renal failure after nephrotoxic nephritis in rats studied by renal transplantation

A. M. EL NAHAS*, R. LECHLER, S. N. ZOOB AND A. J. REES

MRC Clinical Immunology Research Group, Renal Unit, Department of Medicine, Royal Postgraduate Medical School, Hammersmith Hospital, London

(Received 3 May/25 June 1984; accepted 4 July 1984)

Summary

1. We studied the relation between immunopathology and progressive renal failure after nephrotoxic nephritis (NTN) in rats.

2. Thirty days after induction of nephritis by injection of rabbit anti-rat nephrotoxic serum, pairs of kidneys from 13 nephritic rats were transplanted into separate syngeneic recipients, one of whom had been pre-immunized with rabbit immunoglobulin G (IgG) whilst the other was naive.

3. Progression to renal failure of the transplanted nephritic kidney was studied after removal of the recipient’s own kidneys; results from right and left kidney from a single donor in pre-immunized and naive recipients were compared.

4. There were substantial differences in autologous anti-rabbit IgG titres in naive and pre-immunized recipients; despite this pairs of kidneys from the same donor had almost identical courses as assessed by proteinuria, serum creatinine and graft survival.

5. There was substantial variation in survival of kidneys from different donors. But there were very strong correlations of graft survival with proteinuria ($r = 0.97$, $t = 4.443$, $P < 0.001$) and reciprocal serum creatinine ($r = 0.95$, $t = 4.32$, $P < 0.001$) in donors shortly before transplantation.

6. We conclude that autologous antibody titres did not influence the progression to renal failure after nephrotoxic nephritis. The rate of progression was already determined at the time of transplantation.

Key words: chronic renal failure, glomerular sclerosis, nephrotoxic nephritis, renal transplantation.

Abbreviations: FCA, Freund’s Complete Adjuvant; GBM, glomerular basement membrane; IgG, immunoglobulin G; NTN, nephrotoxic nephritis; NTS, rabbit anti-rat GBM antiserum.

Introduction

Progression of glomerulonephritis to terminal renal failure often follows a relentless course, but the rate varies considerably from patient to patient [1]. Glomerular and tubulo-interstitial scarring invariably accompany this progression [2] but the mechanisms responsible are poorly understood. It is not known whether the immunopathology originally responsible for the nephritis must persist for glomerulosclerosis to continue, or alternatively whether scarring can proceed independently.

Before these possibilities can be distinguished, the underlying immunopathology, for example autoantibody or immune complex, must be known and ways to quantify its activity developed. Presently, in man this can be done only for patients with anti-glomerular basement membrane (GBM) antibody mediated nephritis. In these patients reports of serial measurements of anti-GBM antibody titres have documented progression
to renal failure when circulating anti-GBM antibodies are no longer detectable [3]. This suggests that scarring is not invariably linked to the original immunopathology.

Nephrototoxic nephritis (NTN) is an experimental analogue of anti-GBM antibody mediated nephritis. In the telescoped model of this disease acute injury is caused by binding of autologous anti-immunoglobulin G (IgG) antibodies to heterologous IgG fixed to the GBM [4]. Chronic nephritis develops as the acute inflammation is subsiding but the relation of autologous antibody titres to scarring has not been investigated.

The purpose of this study was to assess the feasibility of transplanting both kidneys from a nephritic rat into separate syngeneic recipients, then to use this approach to investigate the relations between immunopathology and progressive renal failure after nephrototoxic nephritis.

Materials and methods

Animals

Syngeneic male Lewis rats weighing 250 g were studied. They were housed up to six per cage with free access to food and water. Once a week they were placed in individual metabolic cages for 24 h urine collections.

Telescoped model of NTN

Rats were immunized with 1 mg of rabbit γ-globulin in Freund's Complete Adjuvant (FCA). Five days later they were injected with 2 ml of rabbit anti-rat GBM antiserum (NTS). This antiserum was prepared as described by Bhan et al. [5]. As natural history of the nephritis varies considerably from experiment to experiment, three control rats from this experiment were followed without transplantation for 120 days.

Renal transplantation (Fig. 1)

Both kidneys were removed from rats 30 days after induction of NTN and transplanted into separate syngeneic recipients. Donor kidneys, whether right or left, were transplanted into the recipient's left renal fossa after removal of the left kidney. Renal artery, vein and ureter were anastomosed end to end. Warm ischaemia times were similar for all pairs of kidneys and never exceeded 45 min. One week after transplantation the recipient's contralateral (right) kidney was removed leaving only the engrafted nephritic kidney, whose function could then be followed. All the surgical procedures were done under general anaesthesia with ether. As controls, normal left kidneys from four rats were transplanted orthotopically into syngeneic recipients, whose contralateral kidney was removed 1 week later. The progress of these normal kidneys was followed for 90 days.

Renal transplant recipients

Both kidneys from a single nephritic donor were transplanted into separate syngeneic recipients. One recipient had been immunized subcutaneously with 1 mg of rabbit γ-globulin in FCA 35 days before transplantation and 5 days later was injected intravenously with 2 ml of normal rabbit serum. This was done to mimic the immunization of the donor but without causing nephritis. The recipient of the other nephritic kidney was not pre-immunized. Both groups of recipients contained similar numbers of rats transplanted with right and left kidneys.

Assessment of nephritis

Weekly measurements were made of rats' weight, urine and serum creatinine concentrations, and 24 h protein excretion. Rats were bled from the tail artery under ether anaesthesia. Creatinine was measured by a standard autoanalyser technique and protein by the biuret method. At death renal morphology was assessed by using standard 0 to 4 scoring systems for glomerulosclerosis and tubular atrophy.
Progression of nephrotoxic nephritis

Measurement of rat anti-rabbit IgG antibody titres

Autologous anti-rabbit IgG antibody titres were measured weekly by solid phase radioimmunoassay. Ninety-six well microtitre plates were incubated with 100 μl of rabbit IgG at a concentration of 100 μg/ml for 3 h at 30°C. After extensive washing, 100 μl of test serum at a dilution of 1:500 was added and incubated for 1 h. After further washing 125I-labelled rabbit anti-rat IgG was added and used to measure the amount of antibody binding. The results were expressed as percentage binding of a reference positive serum. Normal rat sera bound 21 ± 5% (mean ± SD). The upper limit of the normal range was considered to be 31%.

Statistical analysis

Results were expressed as means ± standard deviations (SD). Comparison of autologous anti-rabbit IgG antibody titres were by the Mann-Whitney test. Correlations of results from right to left kidneys from the same donor were made by linear regression using the least squares method.

Results

Natural history of nephrotoxic nephritis

All the rats in this experiment developed evidence of glomerulonephritis shortly after injection of NTS; proteinuria rose to 106 ± 44 mg/24 h (mean ± SD) and serum creatinine from 40 ± 12 μmol/l to 67 ± 20 μmol/l over the first 5 days. After the first 5 days rats continued with stable renal function for at least 30 days until renal transplantation. At this stage there was considerable variation in proteinuria which ranged from 49 to 241 mg/24 h whilst serum creatinine ranged from 45 to 150 μmol/l. Renal biopsies taken from four rats at transplantation showed moderate glomerulosclerosis and mild tubular atrophy. Three control rats with nephritis who were not transplanted continued to have stable renal function until between 60 and 90 days, when serum creatinine and proteinuria both rose (Table 1). Two of these rats died from renal failure in less than 120 days. At autopsy their kidneys showed extensive glomerulosclerosis and tubular atrophy.

Transplantation of nephritic kidneys

Thirteen pairs of kidneys from nephritic rats were transplanted 30 days after induction of nephritis. Five recipients, each from separate donors, died during the transplantation: four from...

| TABLE 1. Serial values for serum creatinine, proteinuria and survival after nephrotoxic nephritis followed by transplantation on day 30 (group I), nephrotoxic nephritis only on day 30 (group II), or transplantation only on day 30 (group III). |
|-----------------|-----------------|-----------------|-----------------|-----------------|
|                  | Creatinine (μmol/l) | Protein (mg/24h) | Survival        |                  |
|                  | Day 0            | Day 60           | Day 90          | Day 120         |
| Group I          |                  |                  |                  |                  |
| NTN Survival     |                  | 66 ± 23          | 66 ± 23         |                  |
| Group II         |                  |                  |                  |                  |
| NTN + transplantation | 68 ± 20 | 68 ± 10          |                  |                  |
| Group III        |                  |                  |                  |                  |
| transplantation  |                  |                  |                  |                  |
|                  |                  |                  |                  |                  |

* Creatinine = serum creatinine (μmol/l), + protein = proteinuria (mg/24h); results expressed as mean ± SD. IIa, Pre-immunized recipients; Iib, naive recipients.
uncontrolled bleeding from transplant biopsies, a procedure then discontinued, and one from primary graft non-function. This left eight pairs of recipients for long-term studies.

Until removal of the recipients' right kidney, proteinuria in transplanted rats was 38 ± 4 mg/24 h, which was appreciably less than that of the donors. However, after contralateral nephrectomy proteinuria from the single nephritic kidneys increased rapidly to 130 ± 16 mg/24 h, which was similar to the amount excreted by donors before transplantation.

**Progression of NTN after transplantation**

Eight pairs of nephritic kidneys were available for long-term study. Two rats from separate pairs died without renal failure: one on day 55 during an anaesthetic for blood sampling and the other on day 60 from an infection. Six pairs were followed until both recipients died from renal failure or were killed at the end of the experiment on day 150.

The rate at which renal failure developed varied considerably, but 11 of the 14 remaining recipients died from renal failure within 120 days of the transplantation. Despite this variability, kidneys from the same donors behaved almost identically whether assessed by measurements of serum creatinine \((n = 33, r = 0.962, t = 3.61, P < 0.001)\) (Fig. 2), proteinuria \((n = 37, r = 0.938, t = 3.57, P < 0.001)\) (Fig. 2) or survival \((n = 6, r = 0.982, t = 6.14, P < 0.001)\) (Fig. 3).

Biopsies taken at autopsy showed the transplanted nephritic kidneys had diffuse glomerulosclerosis with extensive tubular atrophy and interstitial calcification. Immunofluorescent examination revealed rabbit and rat IgG fixed linearly to the GBM of remaining capillary loops. These changes were identical with those found in rats dying after straightforward NTN.

**Autologous antibody response**

All pre-immunized recipients had high titres of anti-rabbit IgG antibodies immediately before transplantation (mean ± SD; 71 ± 6%) which were similar to those of the donors (66 ± 3%). These titres did not vary throughout the course of the experiment (Fig. 4). Sera from naive recipients had background binding of 21 ± 4% before transplantation. However, seven of nine developed low titres of autologous antibody. Maximal titre for this group was 30 ± 5%, which was significantly lower than that of pre-immunized rats \((P < 0.025\) Mann-Whitney).
Progression of nephrotoxic nephritis

Transplantation of normal kidneys

Four normal kidneys were transplanted into syngeneic rats to study the effect of transplantation on renal function and morphology. All four kidneys functioned normally until rats were killed at the end of the experiment. After 90 days their serum creatinines were $60 \pm 4.5$ μmol/l and proteinuria was $19.7 \pm 3$ mg/24 h. Morphological examination revealed hypertrophic glomeruli but no glomerulosclerosis or tubular atrophy.

Influences on transplant survival

The severity of the acute injury in the first 5-10 days after injection of NTS was a poor indicator of the fate of the subsequent transplant. There was no correlation between the maximal early serum creatinine and transplant survival. Although early proteinuria correlated with transplant survival ($n = 11$, $r = 0.87$, $t = 4.43$, $P < 0.001$), the shallow slope of the linear regression equation means that these observations have little predictive value compared with the findings immediately before transplantation. At this stage there were exceptionally strong correlations between the reciprocal of serum creatinine and transplant survival ($n = 12$, $r = 0.953$, $t = 4.31$, $P < 0.001$), and between proteinuria and transplant survival ($n = 11$, $r = 0.966$, $t = 4.43$, $P < 0.001$) (Fig. 5).

These results show that more than 90% of the variation in transplant survival could be predicted before transplantation and thus occurred independently of the environment in which chronic renal failure developed.

Discussion

The understanding of the mechanisms involved in progressive glomerulonephritis is important. In this study we transplanted pairs of kidneys from rats with NTN into syngeneic recipients, then used this approach to explore the relation between immunopathology, acute injury and the subsequent development of chronic renal failure. The principal findings were firstly that large differences in autologous antibody titre during the chronic phase do not influence the rate of progression to renal failure, and secondly that differences between the kidneys at the time of transplantation accounted for the variability of graft survival. But before discussing these findings it is important to consider the validity of our experimental approach.

Matching groups of animals presents considerable problems in long-term controlled studies of experimental glomerulonephritis. Variability in the severity of nephritis after identical treatment is considerable, even in syngeneic animals. This was shown by the range of serum creatinines and protein excretions of our nephritic donors before
transplantation. In the absence of renal artery stenosis [6], the severity of nephritis should be identical in right and left kidneys in a single animal. Potentially these kidneys could be used to provide perfectly matched pairs if transplanted into separate syngeneic recipients. In our study right and left kidneys from the same donor per-
formed almost identically in separate recipients. This demonstrates the feasibility of our approach because the operative procedure did not itself introduce unacceptable variables.

A second, potentially confounding, problem is whether the progressive renal failure we observed in the transplants was caused by the nephritis, or by transplantation per se. This is a serious question as progressive glomerulosclerosis develops in ageing rats, and is accelerated by the reduction of renal mass by nephrectomy [7]. In this study transplanted normal kidneys did not develop pathological proteinuria or glomerular sclerosis during the course of the experiments, whilst in other experiments (unpublished work) we have found that unilateral nephrectomy does not alter the findings in progressive NTN. Thus it seems that our experimental approach is a valid way to study progressive glomerulonephritis in a controlled fashion.

This is useful because transplantation is the only way to assess the influence of large differences in autologous antibody titre without chemical immunosuppression [8]. Our experiments do not preclude a permissive role for such antibodies as low titres developed in seven of nine naive re-
cipients. However, similar performance of paired kidneys shows that the titre of these antibodies has little or no influence on the rate of progression. Furthermore the differences in outcome we observed were predictable before transplantation. The exceptional correlation between measures of the donor’s kidney function immediately before transplantation excludes a role for other systemic factors during the chronic phase, at least under our experimental conditions.

These results suggest that progressive renal failure after acute nephritis can proceed independently of the original immunopathology. But presently we can only speculate on the mechanisms involved. One possibility that has been suggested is that proteinuria itself is nephrotoxic [9]. This is unlikely to explain our results because in separate experiments we have shown that feeding rats with NTN high protein diets increases proteinuria fourfold whilst protecting the kidneys against development of renal failure [10]. Proteinuria increases shortly before the serum creatinine rises after NTN [10, 11] and after sub-total nephrec-
tomy [12]. Possibly it reflects compensatory hyperperfusion and hyperfiltration thought to be nephrotoxic in other models of chronic renal failure [13].

Irrespective of the mechanisms involved, there are striking similarities between our results and the development of chronic renal failure in patients with nephritis. Progression to renal failure after acute post-streptococcal nephritis [14] and anti-GBM disease [3] can occur years after the acute, immunologically mediated, events have subsided. In patients treated for crescentic nephritis renal function during the acute phase injury provides a poor guide to the eventual prognosis [15], whereas the level of renal function immediately after treatment is a much better indicator of the long-
term outcome [16].

It is important to know whether a similar dissociation between the immunopathology and the subsequent development of renal failure is a more general phenomenon. If so it would sub-
stantially affect the approach to the treatment of chronic glomerulonephritis.

Acknowledgments

We thank Miss Sue Goodwin for preparing the manuscript. This work was supported by the National Kidney Research Foundation, whose help is gratefully acknowledged.

References

Progression of nephrotoxic nephritis


