Adriamycin nephropathy in severe combined immunodeficient (SCID) mice

Vincent W. S. Lee1, Yiping Wang1, Xiaohong Qin1, Ying Wang1, Guoping Zheng1, Deepika Mahajan1, Jason Coombes1, Gopala Rangan1, Steven I. Alexander2 and David C. H. Harris1

1Centre for Transplantation and Renal Research, the University of Sydney at Westmead Millennium Institute and 2Centre for Kidney Research, the Children’s Hospital at Westmead, Sydney, Australia

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Introduction

Experimental focal glomerulosclerosis is a model of chronic proteinuric renal disease that has been induced in both rats and mice. In mice, the adriamycin (ADR)-induced nephropathy model is a robust experimental analogue of human focal glomerulosclerosis [1]. Renal injury in the ADR-induced nephropathy model is characterized by changes in both the tubulointerstitial and glomerular compartments. Within the interstitium, there is an increase in interstitial volume, tubular atrophy, collagen deposition and increased numbers of CD4+ T cells, CD8+ T cells and macrophages [1]. In mice with established adriamycin nephropathy, depletion of CD4+ T lymphocytes worsens glomerular and interstitial injury [2] whilst depletion of CD8+ T lymphocytes ameliorates disease [3]. Therefore, lymphocytes play a pivotal role in renal injury in adriamycin nephropathy (AN).

The tubulointerstitium also contains other inflammatory cells such as macrophages, dendritic cells and NK cells. Using lymphocyte-deplete animals enables study of the individual contribution of these cell populations to renal injury. SCID (severe combined immunodeficient) mice are homozygous for an autosomal recessive mutation that leads to the absence of lymphocytes and hypogammaglobulinaemia [4], and are found in BALB/c mice. Adriamycin nephropathy can be induced in immunocompetent BALB/c mice [1]. Previous studies in our laboratory have shown that NK cells do not play a significant role in this model [5].

The aim of the present study was to determine if the model of AN could be established in SCID mice. We found that tail-vein injection of adriamycin induces a stable and reproducible model of proteinuric renal disease in lymphocyte-deplete BALB/c mice at half the dose required for immunocompetent BALB/c mice.

Subjects and methods

Male SCID BALB/c mice weighing 20–25 g and aged 8 weeks were obtained from the Animal Resources Centre, Perth (Australia). Dose-finding studies defined an optimal dose of 5.3 mg/kg body weight of ADR (Adriamycin/C213, Doxorubicin Hydrochloride; Pfizer Australia Pvt Ltd), compared with 9.8 mg/kg in immunocompetent BALB/c mice. ADR was injected once via the tail vein of each non-anaesthetized mouse. Age-matched control SCID mice were injected with an equal volume of isotonic saline. All mice were housed in pathogen-free conditions using standard animal cages with free access to standard chow. All mice were weighed twice daily. An overnight urine collection of spontaneously voided urine was made from each animal on the night before sacrifice. Five mice treated with ADR were sacrificed at weeks 1 and 2. Seven mice treated with ADR were sacrificed at weeks 4 and 6. An equal number of control mice were used at each time point. Blood samples for serum albumin and creatinine (Cr) were obtained by substernal cardiac puncture under anaesthetic (ketamine/xylazine 200/16 mg/kg body weight) and then sacrificed by pneumocardiectomy. Kidney weight and body weight were measured. All kidney specimens were processed without delay.

A piece of kidney tissue was placed in 10% neutral buffered formalin fixative at room temperature for 12 h, embedded in paraffin, cut in 3 μm sections and stained using haematoxylin/eosin and periodic acid-Schiff.

Quantitative analysis of glomerular capillary tuft area, mesangial matrix area, glomerular cellularity, tubular
atrophy and interstitial volume was performed as described previously [6]. These analyses were performed with image analysis software (ImageJ, NIH).

For electron microscopy, 1 mm kidney sections were fixed in modified Karnovsky fixative. Sections were rinsed in 0.1M MOPS buffer and postfixed in 2% buffered osmium tetroxide. Using an automatic tissue processor (Lynx, Vision Biosystems Ltd, Australia), sections were dehydrated in ethanol and infiltrated with epoxy resin. Blocks were embedded and polymerized at 70°C for 10 h. Ultrathin sections were stained with uranyl acetate in 50% ethanol followed by lead citrate, then examined using a Philips CM120 electron microscope.

For immunohistochemical staining, coronal slices of each kidney were placed in OCT embedding compound (Tissue-Tek, Sakura Finetek, USA), frozen in isopentane pre-cooled to −20°C, and stored at −80°C. Frozen sections, 7μm thick, were cut with a cryostat and air-dried at room temperature. Slides were fixed with acetone at −20°C and then air-dried for 30 min. A series of blocking steps using 0.3% hydrogen peroxide, avidin and biotin solution (Dako Australia), and Background Terminator (Biocare Medical, USA) eliminated background staining. The slides were then incubated with the primary antibody rat anti-mouse F4/80 (1:200 dilution) (eBiosciences, San Diego, USA), anti-L3T4 (1:50) and anti-Ly-2 (1:50) (BD Biosciences Pharmingen, USA) overnight at 4°C.

Fig. 1. Functional markers of renal injury in ADR-injected and control mice. Mice injected with adriamycin developed hypercreatininaemia, proteinuria and hypoalbuminaemia, whilst both body and kidney weight fell. Data are mean ± SD. *P < 0.05 **P < 0.005 for comparisons between ADR-injected and control mice at similar time points. ADR (black bar); control (grey bar).
The secondary antibody, polyclonal biotinylated rabbit anti-rat immunoglobulin (1:300 dilution) (DakoCytomation, USA) and streptavidin/peroxidase complex (Vectra Stain kit; Vectra Laboratories, UK) were each incubated for 30 min. DAB chromogen (DakoCytomation, USA) was used to stain the end-products brown. An irrelevant isotype control antibody was used in place of the primary antibody as a negative control. Sections were counterstained with Harris haematoxylin, dehydrated with graded ethanol and histoclear and coverslipped.

Quantitative analysis of macrophage staining was undertaken. Images of sections taken at 400× magnification from eight non-overlapping fields were analysed. Cells that stained brown and were nucleated were counted.

Approval for the study was obtained from the institutions’ animal ethics committee (Western Sydney Area Health Service, Sydney).

All statistical analyses were performed using JMP software version 5.1 (SAS Institute, Carey, NC, USA). Results from the ADR and control groups were compared with each other at each time point using the student’s unpaired t-test.

Results

All SCID BALB/c mice treated with ADR developed nephrotic syndrome and remained alive throughout the study period of up to 6 weeks.

General characteristics

All experimental animals developed nephropathy (Figure 1). The surfaces of all kidneys in ADR-treated animals were paler than in controls. Kidney weight progressively decreased. Mice injected with ADR had functional signs of proteinuric renal disease, including hypercreatininaemia, impaired Cr clearance, hypoalbuminaemia and proteinuria. Body weight fell within the first 2 weeks, reaching a nadir by day 15, then steadily rose but did not achieve baseline. There was no evidence of other organ involvement or carriage of pathogens.

Morphometric and histopathological studies

ADR-injected mice demonstrated progressive histological injury during the 6 weeks after injection of adriamycin (Figure 2). In our quantitative analysis (Figure 3), we observed an initial phase of glomerular hypercellularity but by week 4–6 glomeruli were hypocellular and displayed marked sclerosis. Glomerular tuft size remained similar between AN mice and control mice. Tubular cells became much flatter with time in AN-treated animals suggesting progressive tubular cell atrophy. Tubular diameter was less than in control stages of disease (tubular atrophy), but greater by week 6 (tubular dilatation). Interstitial volume progressively increased.

Electron microscopical studies

ADR-injected mice developed foot process effacement 2 weeks after adriamycin injection, and by 6 weeks the basement membrane was denuded due to loss of foot processes (Figure 4).

Immunohistochemistry

Controls had few macrophages within the tubulointerstitium. As early as 2 weeks after ADR injection, we observed marked macrophage infiltration within the intertubular spaces associated with tubular damage (Figure 5, colour images available in supplementary material). By quantitative analysis, macrophage numbers were three to four times control mice at time points 2, 4 and 6 weeks after ADR injection (Figure 6). In contrast, there were few if any CD4+ nor CD8+ staining cells in kidneys in both ADR-injected and control mice.

Discussion

In this study, we have characterized a reproducible and stable model of proteinuric renal disease in SCID mice. This model was induced by tail vein injection of adriamycin at a dose of 5.3 mg/kg, half that is required in immunocompetent mice. Despite lacking lymphocytes, SCID mice developed functional and histological changes similar to those in immunocompetent mice [1,7].

There are several possible explanations for the increased sensitivity of SCID mice to adriamycin-induced injury. Firstly, SCID mice may lack CD4+ T lymphocytes that protect against adriamycin-induced renal injury. We demonstrated that few if any CD4+ cells were present in kidneys of SCID mice, with or without adriamycin injection. Our laboratory has previously shown that depletion of CD4+ T cells in BALB/c mice...
leads to aggravation of adriamycin-induced renal injury [2], whilst reconstitution of BALB/c mice with FoxP3 expressing CD4+CD25+ T cells ameliorates adriamycin-induced renal injury [[8] and submitted]. The second possibility is that the pharmacokinetic behaviour of adriamycin in SCID is different to normal mice. No study to our knowledge directly addresses this issue in SCID mice, however Johansen [9] found that adriamycin, injected intravenously, accumulated significantly more within the kidney in nude mice (which lack a thymus) compared with normal mice. It is possible that a higher concentration of adriamycin within the kidneys of SCID mice accounts for their greater sensitivity to injury. The third possibility is that genetic variation contributed to the greater sensitivity of SCID mice to

Fig. 3. Quantitative analysis of histological markers of renal injury in ADR-injected and control mice. Glomerulosclerosis, tubular atrophy, tubular dilatation, and interstitial volume expansion were all significantly worse in adriamycin-injected animals compared with controls. Data are mean ± SD. **P < 0.005 for comparisons between ADR-injected and control mice at similar time points. ADR (black bar); control (grey bar).
renal injury (compared with wild-type BALB/c mice). We believe that this is not the case as the mice that we used were SCID mice on a pure BALB/c background. In this model, renal injury may be due to the participation of other immune cells such as macrophages and NK cells. In this study, macrophage infiltration was observed in adriamycin nephropathy in SCID mice. NK cells have previously been shown not to play a significant role in this model [5].

Shappell and colleagues [10] described another model of innate immune renal injury in SCID mice. Unilateral ureteric obstruction (UUO) induced tubulointerstitial injury in SCID mice similar to immunocompetent mice over a 30 day time period. As with our model, renal injury was characterized by marked macrophage infiltration in the ligated kidney, similar to UUO in wild-type mice.

By producing renal injury in a lymphocyte-deplete animal, this model will allow further investigation into the role of innate immune cells such as macrophages in the pathogenesis of chronic proteinuric renal disease.

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Conflict of interest statement. None declared.

References

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