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Research Article

The pathogenesis of aminonucleoside of puromycin (PA) nephrotic syndrome in rats was studied using renal transplantation to separate systemic from renal factors. The nephrotic syndrome was transferred by transplantation of kidneys from rats with established proteinuria. Bilaterally nephrectomized normal rats receiving kidneys removed as early as 15 min after intravenous PA injection (100 mg/kg) of donors also developed proteinuria (602 ± 125 mg/24 hr) and a nephrotic syndrome after the usual induction period of 4-7 days observed in this disease. Arterial perfusion of isolated kidneys with PA (50 mg/kg) followed by perfusion with isotonic saline 3 min later and then transplantation to normal bilaterally nephrectomized rats led to a nephrotic syndrome. Urine protein excretion was 494 ± 42 mg on the 7th day after transplantation. In contrast, urine protein excretion after transplantation of normal kidneys to normal bilaterally nephrectomized rats was 40 ± 20 mg on the 7th day. Exposure of a normal kidney to a nephrotic host environment by transplantation of a normal kidney to a unilaterally nephrectomized PA-injected rat did not transfer the disease to the normal kidney. After removal of the nephrotic kidney 11-13 days after transplantation, proteinuria of the donor kidney was normal (21 ± 13 mg on day 15). These studies indicate that pathogenesis of aminonucleoside nephrosis involves programming of the kidney directly by PA within minutes after exposure although increased urinary protein [...]

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Transfer of Aminonucleoside Nephrosis by Renal Transplantation

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ABSTRACT The pathogenesis of aminonucleoside of puromycin (PA) nephrotic syndrome in rats was studied using renal transplantation to separate systemic from renal factors. The nephrotic syndrome was transferred by transplantation of kidneys from rats with established proteinuria. Bilaterally nephrectomized normal rats receiving kidneys removed as early as 15 min after intravenous PA injection (100 mg/kg) of donors also developed proteinuria (602 ± 125 mg/24 hr) and a nephrotic syndrome after the usual induction period of 4–7 days observed in this disease. Arterial perfusion of isolated kidneys with PA (50 mg/kg) followed by perfusion with isotonic saline 3 min later and then transplantation to normal bilaterally nephrectomized rats led to a nephrotic syndrome. Urine protein excretion was 494 ± 42 mg on the 7th day after transplantation. In contrast, urine protein excretion after transplantation of normal kidneys to normal bilaterally nephrectomized rats was 40 ± 20 mg on the 7th day. Exposure of a normal kidney to a nephrotic host environment by transplantation of a normal kidney to a unilaterally nephrectomized PA-injected rat did not transfer the disease to the normal kidney. After removal of the nephrotic kidney 11–13 days after transplantation, proteinuria of the donor kidney was normal (21 ± 13 mg on day 15). These studies indicate that pathogenesis of aminonucleoside nephrosis involves programming of the kidney directly by PA within minutes after exposure although increased urinary protein excretion does not occur until several days later.

INTRODUCTION

Our recent clinical demonstration of recurrence of idiopathic nephrotic syndrome after renal transplantation

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indicates that circulating factors may be important in the pathogenesis of this disease (1). The renal morphology in the nephrotic syndrome produced in rats by the administration of the aminonucleoside of puromycin (PA)¹ is similar to that of the human disease; at onset the only major glomerular abnormality is fusion of epithelial cell foot processes (2). Renal transplantation was employed in the present study in order to define: the roles of host and renal mechanisms in the pathogenesis of the disease, the relationship of proteinuria to duration of exposure of the kidney to aminonucleoside, and the effects of exposure of a normal kidney to a "nephrotic" environment.

METHODS

Inbred Lewis rats obtained from Microbiological Associates, Inc., Bethesda, Md. and Sprague-Dawley rats (250–300 g) obtained from Simonsen Laboratories, St. Paul, Minn. were housed in metabolic cages and 24-hr urine collections were obtained daily. Aminonucleoside of puromycin, 6-dimethylamino-9-[3'-amino-3'-deoxyriboseyl] purine (ICN Nutritional Biochemicals Div., Cleveland, Ohio) was given in a single dose (10 mg/ml in isotonic saline). Transplantation of kidneys to either bilaterally or unilaterally nephrectomized recipients of the same strain was performed using the technique of Lee (3). Each experimental group contained three or more transplants within a single strain. Control observations included transplantation of kidneys from normal rats to bilaterally nephrectomized rats and unilateral nephrectomy of PA-injected rats. Quantitative 24 hr urine protein excretion before and after transplantation was determined using the Biuret method (4). At the time of transplantation and sacrifice, portions of donor and recipient kidneys were fixed in buffered formalin, sectioned, and stained with hematoxylin-eosin and periodic acid-Schiff. Other portions were rapidly frozen in isopentane prechilled in liquid nitrogen, sectioned in a cryostat, and stained with fluorescein-conjugated monospecific antisera obtained from rabbits injected with rat IgG, β_2C , and fibrin using methods previously described (5). Rat IgG, β_2C , and fibrin were prepared using the methods of Arnason, deVaux St.-Cyr, and Relyveld (6), Mardiny and Müller-Eberhard (7), and Blombäch and Blombäch (8), respectively.

¹ *Abbreviation used in this paper:* PA, aminonucleoside of puromycin.

TABLE I
Transfer of Nephrotic Syndrome with Kidneys from PA-Injected Rats

Experimental group		Number of rats	Strain*	Intravenous dose of PA	Time of transplant after PA injection	Urine protein on day 7 after transplantation
				mg/kg		mg/24 hr + 1 SD
I. Kidney of PA-injected rat transplanted to bilaterally nephrectomized normal rat	(a)	4	SD	150	8-10 days	355±95
	(b)	3	SD	150	48 hr	517±165
	(c)	3	SD	150	24 hr	520±155
	(d)	3	SD	150	4 hr	501±221
	(e)	3	SD	150	1 hr	613±220
	(f)	3	Le	100	15 min	602±125
	(g)	4	Le	50‡	3 min	494±42
II. Unilateral nephrectomy 24 hr after PA injection		4	SD	150	—	657±101§
III. Kidney of normal rat transplanted to bilaterally nephrectomized normal rat	(a)	4	SD	—	—	38±18
	(b)	4	Le	—	—	40±20

* SD = Sprague-Dawley, Le = Lewis.

‡ Renal artery perfusion.

§ 7 days after PA injection.

RESULTS

Transfer of nephrotic syndrome with kidneys from PA-injected rats. The nephrotic syndrome could be transferred when kidneys of rats with an established nephrotic syndrome were transplanted to bilaterally nephrectomized normal recipients on day 8-10 after PA injection. Urine protein excretion of donors was 490±55 mg on the day before transplantation; 4 days later that of recipients was 580±220 mg. Proteinuria had begun to decrease by day 7 after transplantation (Table I, group I a).

Transplantation of kidneys from PA-injected rats during the 4-7 day induction period before the onset of proteinuria (Fig. 1) lead to massive proteinuria of the recipients (Table I, groups I (b-g)). Proteinuria of all groups was comparable to that of rats subjected to unilateral nephrectomy after PA injection but not transplanted (group II). In contrast protein excretion after transplantation of normal donor kidneys of both strains (group III) was less than 60 mg/day during the first 10 days.

Direct arterial perfusion of an isolated kidney with PA followed 3 min later by perfusion with isotonic saline (2 × volume of PA perfusate) and then transplantation of this kidney to a normal recipient also led to massive proteinuria (Table I, group I g) after the usual induction period.

Exposure of a normal kidney to a nephrotic host environment. Recipient rats were injected intravenously with PA (100-150 mg/kg of body weight). After uni-

lateral nephrectomy of the recipient 4 hr later, a normal donor kidney was transplanted to each rat. 11-13 days after transplantation when proteinuria was 582±258 mg/24 hr, the rat's own kidney was removed; the donor kidney was left in place. Urine protein excretion decreased to within normal limits immediately and 4 days later was 21±13 mg/24 hr. In contrast rats initially treated in a similar manner but not subjected to a second nephrectomy had persistent massive proteinuria (610±268 mg on the 15th day after transplantation).

MORPHOLOGIC STUDIES

Glomeruli of all kidneys before and after transplantation appeared histologically normal. Minimal to moderate tubular dilatation was observed in transplanted kidneys from PA-injected rats. Minimal or no tubular dilatation was observed in transplanted kidneys from normal donors. Transplanted kidneys of the outbred (Sprague-Dawley) but not the inbred (Lewis) strain showed moderate interstitial mononuclear cell infiltration. Immunofluorescent studies did not demonstrate glomerular or tubular deposition of IgG, β₂C, or fibrin.

DISCUSSION

The present study indicates that the direct renal effect that leads to massive proteinuria 4 or more days later occurs within minutes after PA injection. Inhibition of PA nephrosis by structural analogues in studies of Derr,

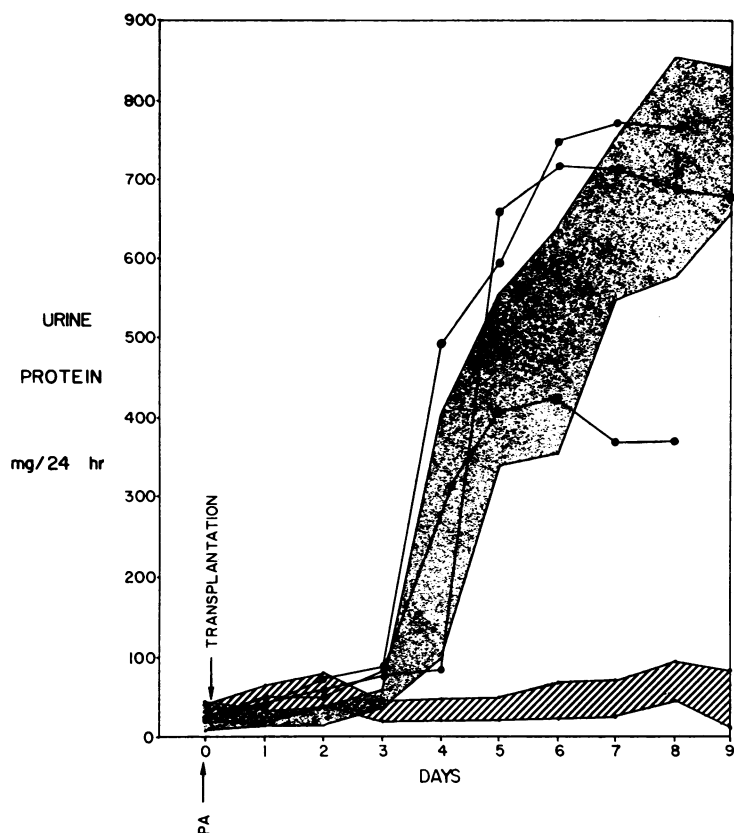


FIGURE 1 Transfer of nephrotic syndrome by transplantation of kidneys from rats given PA (150 mg/kg intravenously): dotted area indicates unilateral nephrectomy of rats injected 24 hr previously with PA (± 1 sd). Renal transplantation after bilateral nephrectomy of normal recipient rats: solid line with circles in indicates donor kidneys from rats given PA 1 hr before transplantation; slanted lines indicate donor kidneys from normal rats (± 1 sd).

Leochler, Alexander, and Nagasawa (9) had suggested the importance of the 1st hr after PA injection.

In contrast to certain instances of human idiopathic syndrome (1), exposure of a transplanted kidney to a nephrotic environment in the present study did not lead to increased protein excretion by the grafted kidney. Thus circulating factors would not appear to be important in pathogenesis of PA nephrosis.

Although the mechanism whereby massive proteinuria is induced by administration of PA is unknown, increased permeability of the glomerular capillary filter to serum proteins has been implicated by increased passage of electron dense proteins across the glomerular basement membrane and epithelial cell membrane barriers (10, 11) and by micropuncture analysis (12). Coincident with increased proteinuria, a striking loss of colloidal iron positive staining of the glomerular polyanion has also been demonstrated in this disease (13).

The present study indicates that the kidney in PA nephrosis is programmed to develop proteinuria very early during the induction period. Systemic and extrarenal metabolic conversion of PA were avoided in this study by direct renal perfusion with PA followed by transplantation. The induction period is apparently not required for extrarenal metabolic conversion of PA since exposure was limited to the transplanted kidney. It is more probable that metabolic turnover of glomerular cell and/or basement membrane constituents in the kidney during this time is required for disruption of the permeability barrier to protein. Studies of Blau and Michael (14) demonstrating a decreased glomerular cell membrane sialic acid content and increased incorporation of glucosamine- 14 C before onset of proteinuria are of interest in this regard. Further metabolic and compositional studies of the glomerulus during the induction phase of PA nephrotic syndrome may lead to improved under-

standing of the pathogenesis of increased glomerular permeability to protein in disease states.

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