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ORIGINAL PAPER

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Elevation of urinary IL-1β levels during transplant associated nephropathy in rat model of the nephrotic syndrome

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Abstract A recurrence of nephrotic syndrome is a well-known phenomenon in patients who receive kidney transplantation. In this study, we attempt to establish a rat model of recurrent nephrotic change after renal transplantation using puromycin aminonucleoside (PA) induced nephrotic rats . We then examine the mechanism leading to recurrence. Female Sprague-Dawley rats (8 weeks) were divided into four groups: group A, allogenic renal transplantation of a normal kidney to PA-induced nephrotic rats; group B, PA injection only as a nephrotic control; group C, allogenic transplantation of a normal kidney to rats receiving a saline injection; group D, rats receiving only a saline injection. The serum and urinary levels of protein, tumor necrosis factor (TNF)-α, interleukin (IL)-1β, IL-2 and interferon (IFN)-γ were measured. The cytokine levels were assessed by performing enzyme-linked immunosorbent assays. Six days after renal transplantation, the kidneys were excised, tissue sections were stained with hematoxylin-eosin and the specimens were studied for pathological alterations. The urinary protein levels and the histological results of protein casts in renal tubules of the autologous kidneys of the nephrotic rats and the transplanted kidneys confirmed the recurrence of nephrotic syndrome in the transplanted kidney. After renal transplantation, urinary protein and IL-1β levels were significantly elevated in recurrent transplanted kidney groups compared with those in the control groups, while the TNF-α, IL-2, and IFN-γ levels were not elevated. In addition, serum levels of TNFα and IL-1β were not elevated. These results suggested that IL-1β may relate to the recurrent nephropathy in the transplanted kidney in the nephrotic rat.

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Introduction

A recurrence of nephrosis with histological evidence of focal glomerular sclerosis (FGS) is a well known phenomenon in patients who receive a kidney transplant [27, 33]. Its incidence ranges from 6% to 38% [26, 28]. The mechanism that governs this syndrome and its association with FGS is not clearly understood [19, 28]. Humoral factors may mediate both the initial and recurrent disease [28]. Several reports have suggested that cellular immunity in conjunction with cytokine production is involved in the pathogenesis of FGS [7, 8, 15]. Animal studies have demonstrated that elevated levels of cytokine can damage the kidneys, and that tumor necrosis factor (TNF)-α, interleukin (IL)-1β, and interferon (IFN)-γ each induce glomerular injury [4, 10, 30]. As previously reported, a model of the minimal change nephrotic syndrome (MCNS) and of FGS induced in the rat by puromicin aminonucleoside (PA), have been established [9]. The present study attempted to evaluate the serum and urinary levels of cytokines (TNF-α, IL-1β, IL-2, and IFN-γ) in PA-related models of rat nephrotic syndrome as well as the recurrence of PA-induced nephropathy in transplanted kidneys. Histopathological studies were also performed to investigate the mechanism of the recurrent disease.

Materials and methods

Nephrotic syndrome rat model

Eight-week-old female Sprague-Dawley rats (Shizuoka Laboratory Animals, Shizuoka, Japan), were used. PA nephrosis as a nephrotic syndrome model was induced by a single intraperitoneal injection of PA (Sigma, St. Louis, Mo., USA) at 15 mg/100 g body weight according to the method of Kokui et al. [24]. PA was dissolved in

saline at a dilution of 20 mg/ml. Control rats received an equal volume of intraperitoneally administered saline.

Renal transplantation

Allogenic kidney transplantation was performed as described by Fisher and Lee [12, 13, 22], and was carried out under pentobarbital anesthesia (50 mg/kg). The left kidney of the normal donor rat was irrigated through the descending vena cava with cold heparinized saline solution and subsequently removed. The excised organ was immediately placed in a cold saline solution while the recipient rat was made ready to receive it. By using 4× loups, the graft artery and vein were sutured to the recipient aorta and the vena cava respectively with end-to-side interrupted 9–0 nylon sutures. Nephrectomy of the autologous kidney of the recipient rat was not performed in order to investigate whether nephrosis in the natural kidney affected the transplanted kidney. The graft ureter was implanted and fixed to the urinary bladder with 9–0 nylon sutures to complete the transplantation procedure.

Experimental design

The following four groups of rats were studied for a total of 16 days (day 0 through day 15, Fig. 1): group A ($n=5$), allogenic renal transplantation of the normal kidney to the PA-induced nephrotic rats was performed on day 9; group B ($n=5$), PA injection without renal transplantation; group C ($n=5$), allogenic transplantation of a normal kidney to saline-injected rats on day 9; group D ($n=5$), saline injection without renal transplantation. PA or saline was injected on day 2 in each group. In each group, 24-h urine was collected on days 1, 3, 5, 7, 10, 12, and 14. All rats in all groups were killed on day 15, with the sera being collected only at this time. In every specimen, the urinary levels of protein and the urine and serum levels of TNF-α, IL-1β, IL-2 and IFN-γ were measured. On day 15, the kidneys were excised for histopathological examination by light microscopicy.

Cytokine assays

Specimens of urine and sera were frozen and stored at –70°C until assayed. Cytokine levels were assessed by performing enzyme-linked immunosorbent assays in duplicate. Immunoassay kits for IL-1β, IL-2 and IFN-γ were provided by BioSource International (Camarillo, Calif.), and the kit for TNF-α was donated by Genzyme (Cambridge, Mass.). For the measurement, the internal standard and control sample for each cytokine was always used when the rat specimens were assayed.

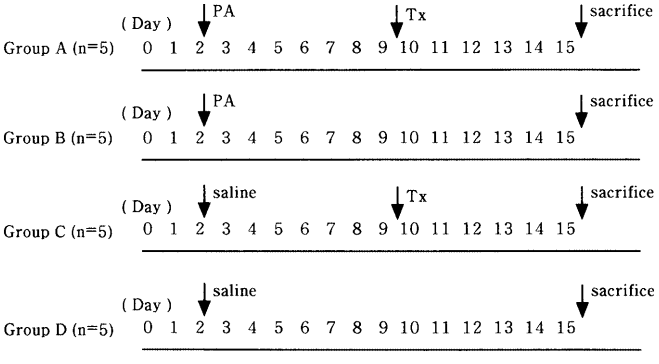

Fig. 1. The experimental design. Urine samples were collected from each rat on days 1, 3, 5, 7, 10, 12 and 14. Sera were collected on day 15 when the rats were killed. *PA*, puromycin aminonucleoside; *Tx*, renal transplantation

Preparation for light microscopy

The grafted and the natural kidneys were excised on day 15. They were cut in half along a longitudinal incision through the hilum, fixed in 10% buffered formalin by immersion, and embedded in paraffin. Tissue sections approximately 4 μm thick were prepared and stained with hematoxylin-eosin.

Statistical analysis

Data were expressed as means ± SD. Differences between the data sets of the groups were compared by performing an analysis of variance (ANOVA). A level of $p < 0.05$ was accepted as statistically significant.

Results

Levels of protein in the urine

Figure 2 shows the 24-h mean urinary protein levels for each group. On day 7 (5 days after PA administration), the mean value for 24-h urinary protein excretion rose to 597 ± 238 mg/day for the rats in group A with renal transplantation, and to 451 ± 56 mg/day for the rats of group B without renal transplantation. By 10 days after PA dosage, the urinary protein levels in the group B rats gradually decreased while those in the group A rats remained at a high level. The differences between the mean urinary protein levels in the rats of groups A and B were statistically significant on both days 12 and 14 ($p < 0.05$). These results suggest that urinary protein was excreted from the transplanted kidney on days 12 and 14. The values for the 24-h urinary protein levels in the rats in groups C and D neither exceeded 6 mg nor increased after renal transplantation in group C.

Levels of cytokines in the urine and serum

The urinary levels of TNF-α in all groups were below the minimum detectable, and the urinary levels of IL-2 and

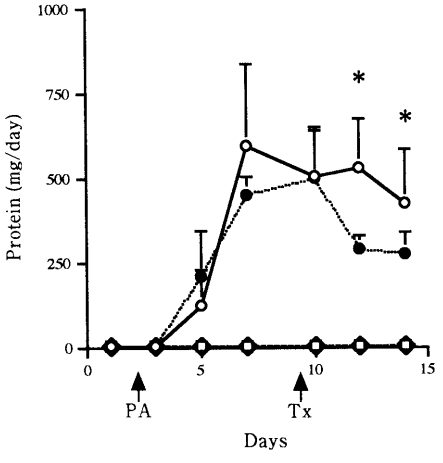

Fig. 2. Urinary protein levels. *Empty circle*, group A; *filled circle*, group B; *empty square*, group C; *empty diamond*, group D. An *asterisk* indicates $P < 0.05$ between groups A and B. *PA*, puromycin aminonucleoside; *Tx*, renal transplantation

IFN-γ in nephrotic animals (groups A and B) did not differ from the corresponding control values (groups C and D). After transplantation, there were no significant difference among the four groups. The level of IL-1β in the urine of the rats in group A (PA nephrosis rat with renal transplantation) was significantly elevated to 524 ± 329 pg/ml and 484 ± 388 pg/ml, on days 10 and 12 after transplantation, respectively, compared with the other groups ($p < 0.05$, Fig. 3). Despite transplantation, no change in IL-1β levels was detected in group C (normal rat with renal transplantation). The serum levels of TNF-α, IL-1β, IL-2, and IFN-γ on day 15 were below the minimum detectable in all rats.

Histopathological findings in the transplanted kidney

Apparent glomerular abnormalities were not observed in the autologous kidneys of the nephrotic rats, but the renal tubules were filled with protein casts (Fig. 4). These findings show that minimal change nephropathy occurred in the rats injected with PA. This is consistent with previous reports [9]. Renal tubular protein casts were also observed in one of the five grafts in the group A rats. This animal exhibited especially high levels of urinary IL-1β (Fig. 5). Conversely the glomerulus and renal tubules of the remaining four grafts in group A did not show any significant pathological changes.

Discussion

The study of cytokines as possible pathogenic factors in the recurrence of disease in the transplanted kidney can increase our understanding of the mechanisms of nephritis. No studies have examined the circulating levels of cytokines in nephrotic rats undergoing renal transplantation, thus evidence for any involvement of cytokines in the pathogenesis of nephrosis is lacking. This study assessed the simultaneous levels of four inflammatory cytokines (TNFα, IL-1β, IFNγ and IL-2) in the urine of rats with an active PA-induced nephrotic syndrome, with or without renal transplantation, to investigate whether the cytokines influenced the recurrence of transplanted nephropathy in the transplanted kidney.

In this experimental model, we have established a recurrent nephropathy model for the transplanted kidney. We have shown that urinary protein was apparently excreted from the transplanted kidney, and that protein casts were histopathologically observed in the renal tubules. These findings revealed that nephropathy recurred in the transplanted kidney.

The importance of the cytokines in renal damage has been demonstrated in animal models. TNF-α, IL-1β, and IFN-γ have been shown to exacerbate proteinuria and to accelerate the course of glomerular injury in experimental nephritis [3, 6, 20, 41]. In addition, the glomerular expression of TNF-α and IL-1β in several

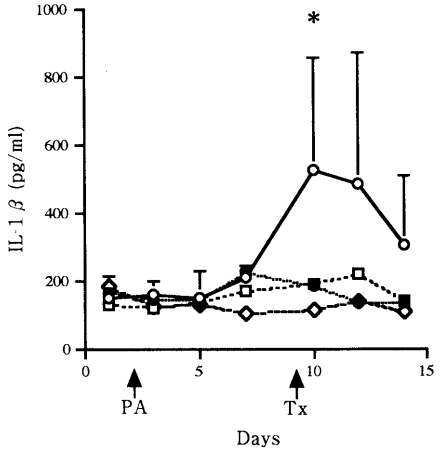

Fig. 3. The levels of IL-1β in the urine. *Empty circle*, group A; *filled circle*, group B; *empty square*, group C; *empty diamond*, group D. An *asterisk* indicates $P < 0.05$ between groups A and groups B, C, or D. *PA*, puromycin aminonucleoside; *Tx*, renal transplantation

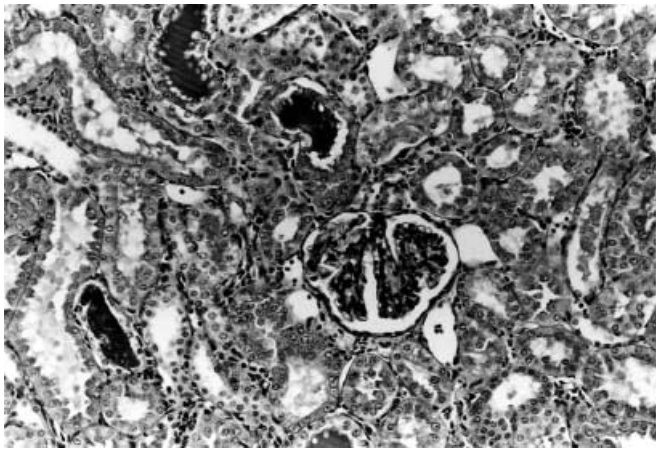

Fig. 4. A tissue section from the autologous kidney removed from a nephrotic rat that received a renal transplant (HE, ×200)

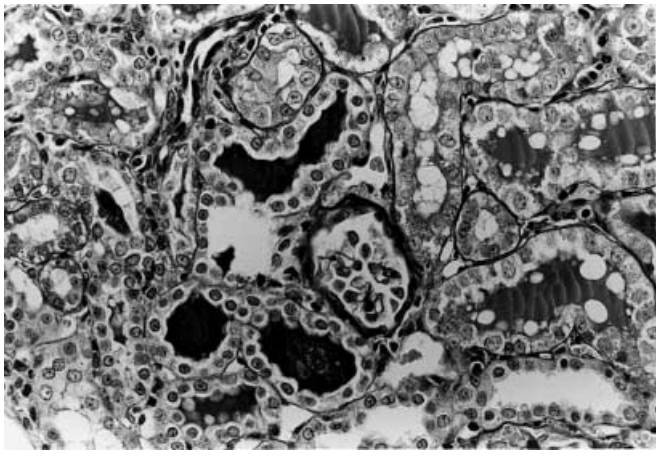

Fig. 5. A tissue section from the graft kidney from a rat with markedly elevated levels of IL-1β (HE, ×200)

types of glomerulonephritis have been reported by the use of in situ hybridization techniques [31, 37, 43]. Thus, TNF-α and IL-1β may be important in the development of glomerulonephritis. In contrast, IL-2 in supernatants of phytohaemagglutinin, stimulated peripheral blood mononuclear cells in vitro were reported to be increased in relapse of minimal change nephropathy [35]. However, no consensus has been arrived at on the role of IL-2 in the causation of the nephrotic state [16, 18, 21, 32, 38].

In this study, we determined the serum levels of cytokines only once because of the poor condition of the rats due to the PA-induced nephrotic syndrome. No elevation of the serum levels of TNF-α or IL-1β was demonstrated. In contrast, a significant elevation of IL-1β levels in the urine was demonstrated in the rats with recurrent nephrosis. The source of elevated levels of IL-1β could not be determined. The source of the IL-1β is likely to be cells of the monocyte/macrophage lineage which are resident in the glomerular mesangium and are frequently observed in the renal interstium of nephrotic subjects [1]. In addition, the contribution of monocytes/macrophages, rather than mesangial cells, to local IL-1 synthesis has been emphasized in studies on animal nephritis models [10, 39, 40]. In acute aminonucleoside nephrosis in rats, an immunohistochemical based correlation was reported between the number of glomerular macrophages and IL-1 positive glomerular cells. IL-1 is chemotactic for neutrophils in vivo [11] and affects neutrophils and other phagocytes in vivo [23, 42]. It can cause acute inflammation [14] and may induce damage in glomeruli. Brennan et al. [6] reported that IL-1 can contribute to nephritis in murine models of lupus. In addition, experiments using a rat model of anti-glomerular basement membrane antibody-mediated nephritis demonstrated that IL-1β can increase the severity of glomerular injury in nephritis [41]. However, the mechanism by which IL-1β could provoke proteinuria remains unknown. Further investigations are necessary to elucidate its mechanism in detail.

On the other hand, the urinary levels of TNF-α, IL-2, and IFN-γ were not elevated. The urinary levels of TNF-α were below the minimum detectable level of our assay. The significance of TNF-α levels in the urine is difficult to interpret because of its incomplete recovery from urine, probably due to inhibitory factors, as shown in preliminary spiking experiments [17, 25, 34]. Because of the difficulties associated with detecting TNF-α, it is necessary to establish more specific and precise methods for measuring TNF-α in urine. Suranyi et al. [36] demonstrated that only TNFα increased in human urine and serum of patients with nephrotic syndrome (FGS and membranous nephropathy), and IL-1β, IL-2, IFNα, or IFNγ were not elevated. In contrast, nephrotic patients with MCNS did not show an elevation of these cytokines. Therefore, differences in the pattern of change of cytokines may be observed dependent on the different status due to the disease. However, the reason why only IL-1β increased in our study is not known.

In addition to cytokines, other humoral factors or local protein factors may also cause nephritis. Several investigators have demonstrated that the infusion of stimulated lymphocyte supernatants from patients with MCNS into rats caused a patchy spreading of the foot processes of epithelial cells, a reduction in the anionic charge of the glomerular basement membrane, and an increase in urinary protein excretion [2, 5, 29, 44]. In our experiment, the urinary secretion of IL-1β may be induced by unknown factors excreted from the nephrotic kidney.

We concluded that renal transplantation in the nephrotic rat model may induce the expression of IL-1β which may induce recurrent nephropathy in the transplanted kidney. In turn, the degree of expression of IL-1β may influence the viability of the graft. The urinary level of IL-1β may be a good clinical marker for monitoring graft function and recurrent disease after kidney transplantation in relation to the nephrotic condition.

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