Recombinant Interleukin-2 Alone Did Not Induce Proteinuria with Changes in Anionic Sites of the Glomerular Basement Membrane in Rats

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Table 1. Time course change of UProE, UFR and UProE/UCrE following intravenous injection of rIL-2 at a dose of 70×10^4 JRU (mean $\pm$ SE, n = 8)

Dear Sir,

Interleukin-2 (IL-2) is a lymphokine modulating T cell function. Recombinant human IL-2 (rIL-2) has been used in adoptive immunotherapy for malignancies. The major adverse effect of rIL-2 treatment is the vascular-leak syndrome, in which vascular permeability is increased, resulting in a leakage of proteinaceous fluids [1]. On the other hand, the production of a vascular permeability factor derived from T lymphocytes has been demonstrated in the minimal change nephrotic syndrome (MCNS) [2,3]. We reported a patient with nephrotic syndrome, which was considered to occur coincidentally with rIL-2 treatment for malignant hemangioepithelioma, and where rIL-2 might cause MCNS [4]. In order to clarify whether rIL-2 might be involved in proteinuria associated with a reduction in anionic sites of the glomerular basement membrane (GBM), we studied the acute and chronic effects of rIL-2 in rats.

Wistar-Kyoto rats (WKY) were cannulated in the right jugular vein and the bladder, and isotonic saline (20 μ l/min) was infused throughout the experiment. Saline or several doses of rIL-2 (Shionogi Pharmaceutical Co., Osaka, Japan) were injected via the jugular vein. Urine samples were collected every 5-10 min. Urine flow rate (UFR), urinary protein excretion (UProE) and urinary creatinine excretion (UCrE) were measured. For the histological study, 0.5% of polyethyleneimine (PEI) was injected as a cationic probe 10 min after the injection of rIL-2 or isotonic saline. UProE did not change after the rIL-2 infusion at doses of 3.5 - 35×10^4 JRU as compared to the level before injection. However, the injection of rIL-2 at a dose of 70×10^4 JRU caused

an increase in UProE by 3-fold in comparison with the control phase and a concomitant increase in UFR by 5-fold. A UProE/UCrE ratio was not different before and after the injection of rIL-2 (table 1). Ten minutes later, these modifications returned to the levels before injection. A dose-dependent increase in UProE was no longer evident in a dose of 140×10^4 JRU of rIL-2. Anionic sites were stained regularly with PEI on the lamina rara externa

and irregularly on the lamina rara interna in the GBM of rats infused with isotonic control saline. This distribution of anionic sites was similarly observed in the specimens obtained from the rIL-2 (140×104 JRU) infused rat kidney (fig. 1).

In the chronic study, WKY received an intraperitoneal injection of the agent at a dose of 35×104 JRU or saline consecutively for 7 days. Twenty-four-hour urine samples were collected throughout the experiment. At the end of the experiment, 0.5% of PEI solution was injected into the jugular vein and then the kidney samples were studied ultrastructurally. UProE gradually increased day by day

both in the rats injected with rIL-2 and saline: rIL-2 group, 3.3 (control) vs. 9.3 (max) mg/day/100 g body weight ($n = 2$); saline group, 2.9 vs. 7.9 ($n = 2$). Differences in UProE and UFR were not evident between the two groups. Histological findings on the distribution of a cationic probe in GBM were similar in the two groups.

While the pathogenesis of MCNS remains enigmatic, several experimental results suggest an involvement of a factor secreted by T cells (a lymphokine) in the disease process [2, 3].

Especially, enhanced IL-2 production has been suggested as a possible mechanism for proteinuria in MCNS [5], even though decreased production and responsiveness of IL-2 in the lymphocytes of patients with MCNS was recently reported [6]. Although rIL-2 increases vascular permeability [1], enhanced proteinuria following rIL-2 is questionable. Our experiments, using intravenous injection of rIL-2 *in vivo*, showed temporal effects on enhancement of proteinuria associated with increased UFR. These findings were similar to those reported by Heslan et al.

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[7]. However, the UProE/UCreE ratio did not change throughout the acute study. Although the etiology of enhanced proteinuria remains unknown, at least a reduction in anionic sites of GBM may not be involved in an exaggeration of proteinuria. Furthermore, chronic intraperitoneal injection of rIL-2 did not affect the anionic sites of GBM and glomerular permeability to protein. These experimental results suggest that rIL-2 itself may not participate in an induction of proteinuria via changes in anionic sites of GBM in rats, nor in a genesis of nephrotic syndrome.

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Fig. 1. In the GBM of the rats, particles of PEI are regularly arranged on the lamina rara externa and irregularly on the lamina rara interna. a Rat with isotonic saline, b Rat with rIL-2. 140×104 JRU. $\times 30,000$.

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