TNF Inhibitory Activity in the Urine of Chronic Renal Failure Patients with Glomerulonephritis

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Dear Sir,

It is supposed that renal diseases, especially glomerulonephritis (GN), are caused by dysfunction of immunological reaction. Recently, a correlation between cytokine levels such as TNF, IL-1, IL-6 and triggering or aggravation of the renal diseases has been reported [1-5], while the existence of urinary and serum inhibitors or binding proteins against cytokines such as TNF or IL-1 and their biological activity have been described [6-8].

In this paper, we examine the TNF inhibitory activity in the urine of both chronic renal failure (CRF) patients with congenital renal diseases and those with GN, in order to determine if TNF and/or TNF inhibitors are related to CRF with GN.

Ten milliliters of urine were freshly obtained from a pool of 10 CRF patients (6 boys, 4 girls, mean age 12.3 years, range 6-18). Two of these were suffering from polycystic kidney, 1 from hypoplasia, 1 from juvenile nephronophthisis, 1 from oligomeganephronia and 5 from GN. Thirty-three healthy children without any inflammatory diseases or renal diseases aged 10.8 years (range 8-16) served as normal controls.

All urines were centrifuged (1,800 g, 5 min) to remove the insoluble materials. Each 2 ml of supernatants was concentrated by ultrafiltration with a membrane of a molecular-weight cut-off of 10 kD (Centricon 10; Amicon Inc.), and four-fold condensed urines were used for bioassay of TNF inhibitory activity. TNF inhibitory activity was measured in an assay of cytotoxicity using the TNF-sensitive cell line L929 [9]. Briefly, 4×10^5 cells/ml in 10% fetal calf serum-RPMI containing 2 µg/ml of actinomycin D was prepared, and 100 µl of cell suspension was inoculated in 96-well microtiter plates. After 2 h of incubation in 5% CO₂ at 37 °C, 50 µl of samples and 50 µl of 2.6 ng/ml recombinant TNF (specific activity 3×10^7 U/mg [10] in 10% fetal calf serum-RPMI) were added to each well, followed by 18 h of incubation in 5% CO₂ at 37 °C. After washing with phosphate-buffered

saline, viable cells were stained by crystal-violet then solubilized with 100 μ l of 0.5% sodium dodecyl sulfate, and absorbance at 595 nm was measured.

The TNF inhibition rate was calculated by the following formula:

$\frac{[\text{OD}_{595}]_{\text{TNF only}}}{[\text{OD}_{595}]_{\text{TNF free}} - [\text{OD}_{595}]_{\text{TNF only}}} \times 100(\%)$.

TNF inhibition rate = $\frac{[\text{OD}_{595}]_{\text{TNF}} + [\text{inhibitor}]}{[\text{OD}_{595}]_{\text{TNF free}} - [\text{OD}_{595}]_{\text{TNF only}}}$

A slight TNF inhibitory activity was found in the urine of healthy children, whereas it was considerably higher in the urine of CRF patients ($p < 0.01$). Among the CRF patients, the urine from the patients with GN showed high percentages of TNF inhibitory activity ($p < 0.05$; fig. 1).

As the functions of TNF have been well characterized in vitro, it is supposed that this cytokine might be involved in acute/chronic inflammatory diseases [11]. In our experiment, the urine from CRF patients with GN, having shown mesangial proliferation and chronic

inflammatory changes, clearly demonstrated a high TNF inhibitory activity. The fact that IL-6 is the mesangial cell growth factor and its production is induced by TNF has been reported [4, 5]. Furthermore, serum and urinary TNF binding proteins in CRF patients have been shown to act as regulators of the bioactivities of TNF [6, 7]. Thus, it is suggested that the urinary excretion of TNF inhibitors in CRF patients with GN might be induced by overproduction of TNF.

Studies about the TNF inhibitor are in progress. From the amino acid sequences deduced from cDNA sequences of TNF receptors, TNF inhibitors in the urine were supposed to be the truncated forms of TNF receptors [12-14]. We have also confirmed that the TNF inhibitory activity in the urine of one patient was caused by two TNF inhibitors. One was derived from a TNF receptor, p55, and the other might be different from a soluble TNF receptor, p75 [unpubl. data]. We are now on the way to purify large amounts of these inhibitors and to clarify the in vivo effects of the inhibitors from the patient.

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Fig. 1. TNF inhibition rate of urine samples from the patients with CRF and healthy controls. The mean standard error of TNF inhibition rate was $5.5 \pm 1.0\%$ in normal controls ($n = 33$, $\bullet$) and $32.0 \pm 7.5\%$ in CRF patients ($n = 10$; $p < 0.01$); in CRF with GN ($n = 5$, $\blacksquare$) and CRF with congenital renal diseases ($n = 5$, $\circ$), it was 46.1 ± 11.1 and $17.4 \pm 4.3\%$, respectively ($p < 0.05$).

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