J Am Soc Nephrol 11: 700–707, 2000

Complement Activation Products in the Urine from Proteinuric Patients

YOSHIKI MORITA, HIROSHI IKEGUCHI, JIRO NAKAMURA, NIGISHI HOTTA, YUKIO YUZAWA, and SEIICHI MATSUO
Division of Nephrology, Internal Medicine III, Nagoya University School of Medicine, Nagoya, Japan.

Abstract. The presence of plasma proteins in the tubular lumen has variety of adverse effects on the tubular cells. Among various plasma proteins filtered through glomerular barrier, complement has been proven as the possible candidate inducing tubulointerstitial injury. To study the role of intratubular complement activation in proteinuric patients, complement activation products (CAP) at C3 level (iC3b and Bb) and C9 level (membrane attack complex) were measured in both plasma and urine of patients with minimal change nephrotic syndrome (MCNS), focal glomerular sclerosis, IgA nephropathy, membranous nephropathy, and diabetic nephropathy. For evaluation of the effect of metabolic acidosis on the intratubular complement activation, urinary CAP were measured before and after sodium bicarbonate administration in patients with renal insufficiency. The following results were obtained: (*1*) Patients with focal glomerular sclerosis and diabetic nephropathy showed the highest level of urinary CAP excretion rate (unit/creatinine), while MCNS revealed no increase. (*2*) Patients with membranous nephropathy showed a unique finding, *i.e.*, isolated increase of membrane attack complex excretion. (*3*) There was no significant correlation between urine and plasma levels of CAP. (*4*) Except for MCNS patients, the urinary excretion rate of CAP significantly increased when the level of proteinuria exceeded the nephrotic range, and it was significantly correlated with the serum creatinine level. (*5*) Urinary CAP excretion rate significantly decreased 2 wk after sodium bicarbonate administration without affecting the level of proteinuria or plasma CAP. These results suggest that the degree of intratubular complement activation correlates with the level of proteinuria, type of glomerular disease, impairment of renal function, and metabolic acidosis.

There is general agreement that the degree of tubulointerstitial injury correlates well not only with renal function at the time of biopsy (1), but also with functional outcome of the kidney (2). On the other hand, decline of GFR has been reported to correlate with degree of proteinuria (3,4). According to a survey by an Italian research group, patients with heavy proteinuria (>3.9 g/24 h) lost kidney function rapidly, and 32.5% reached end-stage renal failure (ESRF) over a median follow-up of 23 mo in patients with nondiabetic proteinuria, while only 4.3% reached ESRF in patients with less proteinuria (<1.9 g/24 h) (5). The presence of plasma proteins in the tubular lumen is thought to be harmful to tubular cells (4,6–16). Complement has been believed to be one of the possible candidates mediating tubular injury in the proteinuric condition (17–23). Camussi *et al.* demonstrated for the first time that tubular deposition of C3 correlated well with urinary C3 excretion in proteinuric patients (24). In both rat and human membranous nephropathy (MN), it was demonstrated that urinary excretion of membrane attack complex (MAC) was markedly increased (25,26). The origin of urinary MAC has been considered the glomerulus, *i.e.*, internalization by glomerular epithelial cells of subepithelial immune complexes containing corresponding antigen, antibody, and MAC, and the subsequent shedding of MAC into urinary space (27). Thereafter, Ogrodowski *et al.* reported the preliminary data that urinary MAC excretion was increased not only in patients with MN, but also in those with focal glomerular sclerosis (FGS) and diabetic nephropathy (DM-N) in which glomerular deposition of complement was not detected (28). He also demonstrated that urinary excretion of MAC was significantly correlated with the degree of proteinuria. He proposed the hypothesis that complement activation and MAC formation were taking place in the tubular lumen in patients with nonselective proteinuria. This notion was tested in the animal models of nephrotic syndrome, and complement activation in the tubular lumen was recognized as a culprit for the proteinuria-associated tubulointerstitial injury (29,30). The mechanisms of intratubular complement activation can be explained by the following facts. First, expression of the cell membrane-associated inhibitors of complement at C3 level (CD46 or MCP: membrane cofactor protein; CD55 or DAF: decay accelerating factor) is hardly detectable in the luminal surface (brush border) of the proximal tubules (31). Second, ammonia (which activates C3 by amidation) is produced in the proximal tubular cells and excreted in the tubular lumen (32,33). The rate of ammonia production is increased in the remnant nephrons or under the presence of proteinuria and metabolic acidosis (34–37). Thus, in protein-

Received June 7, 1999. Accepted August 24, 1999.
Correspondence to Dr. Seiichi Matsuo, Division of Nephrology, Internal Medicine III, Nagoya University, School of Medicine, 65 Tsurumai-cho, Showa-ku, Nagoya 466-8550, Japan. Phone: +81 52 744 2192; Fax: +81 52 744 2209; E-mail: smatsuo@med.nagoya-u.ac.jp
1046-6673/1104-0700
Journal of the American Society of Nephrology

uric conditions, intratubular complement activation is assumed to be taking place via alternative pathways (20,38), and it may be enhanced by the presence of renal insufficiency and metabolic acidosis (39).

The present study was designed to test the hypothesis in the clinical setting that the degree of complement activation in the tubular lumen is closely related to the type of glomerular injury, level of proteinuria, loss of functioning nephrons, and metabolic acidosis in proteinuric patients. Results obtained from this study affirmed the hypothesis, and complement activation in the tubular lumen was further confirmed as the important causative factor in proteinuria-associated tubulointerstitial injury.

Materials and Methods

Patient Profile

Studies were performed in 99 patients with proteinuria including 18 patients with minimal change nephrotic syndrome (MCNS), 10 with FGS, 30 with IgA nephropathy (IgA-N), 24 with MN, 17 with DM-N, and 10 healthy subjects. Patients were being treated at Nagoya University Hospital and its affiliated hospitals. They did not undergo treatment with steroids, immunosuppressive agents, or inhibitors of angiotensin-converting enzyme (ACE) at the time of study. Diagnosis was made based on the finding of renal biopsy, except for 12 patients with DM-N. In the diabetic patients not biopsied, the diagnosis of DM-N was based on the development of persistent proteinuria more than 1 g/d after more than 15 yr of diabetic history and the presence of diabetic retinopathy. All of the patients showed proteinuria more than 1 g/d. Precise data are given in Table 1.

Collection of Urine and Plasma

Nine milliliters of fresh urine was mixed with 1 ml of 10 mM Tris buffer, pH 8.6, with 0.05% Tween 20 and 0.01% of NaN_3 containing protease inhibitors (10 mM benzamidine, 10 mM ϵ-aminocaproic acid, 20 mM ethylenediaminetetra-acetic acid (EDTA), and 100 kallikrein inhibitor units of aprotinin) (28). This mixture was centrifuged at 2000 rpm for 10 min and stored at −70 °C until use. Plasma specimens were taken from blood samples drawn in EDTA at the time of urine collection. EDTA-plasma was also stored at −70 °C until measurement. According to our preliminary study, decay of iC3b, Bb, and MAC as assessed by the enzyme-linked immunosorbent assay (ELISA) was negligible if the above-treated samples were measured within 3 mo after sampling. Thus, all of the urine and plasma specimens were used for the study within 3 mo after collection.

Western Blot Analysis of Urinary CAP

To investigate the difference between the intratubular activation of native C3 molecules and activated C3 fragment leaked from glomeruli, C3 breakdown products in urine samples of various renal diseases was analyzed by Western blot under reduced conditions. Native human C3, C3b, and factor I was purchased from Calbiochem-Novabiochem Corp. (San Diego, CA). C3b was cleaved to iC3b, C3dg, and C3c by factor I and soluble complement receptor type 1 to obtain standard bands (40). Soluble complement receptor type 1 was kindly provided by Yamanouchi Phamaceutical Co. (Tokyo, Japan) and T Cell Sciences (Needham, MA). Urine samples were prepared with dilution for 1 mg/ml concentration and separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and then transferred to a nitrocellulose membrane. After blocking with skim milk, membrane was incubated with peroxidase-labeled goat anti-human C3 (Cappel, Durham, NC). The reaction was visualized using diaminobenzidine and hydrogen peroxide.

ELISA

Complement activation products (CAP), *i.e.*, iC3b, Bb, and MAC, were measured in the urine and plasma using ELISA kits (Quidel Co., San Diego, CA) according to the manufacturer's instructions. Urine and plasma samples were often diluted to obtain concentration for the optimal density reading described in the ELISA kit instructions (the detection limit of the ELISA assay: iC3b 0.21 μg/ml, Bb 0.046 μg/ml, MAC 28.89 ng/ml) (intra-coefficients of variation: iC3b 12.83%, Bb 8.63%, MAC 12.8%) (inter-coefficients of variation: iC3b 16.11%, Bb 9.82%, MAC 13.09%). For urine samples, creatinine and total protein were also measured. The excretion rate of CAP was calculated by the ratio of urinary concentration of CAP to the urinary creatinine

Table 1. Patient profile[a]

Group	*n*	Age	Gender (M/F)	S_{Cr} (mg/dl)	UP/U_{Cr}
Control subjects	10	44.0 ± 2.9 (29 to 56)	5/5	0.74 ± 0.08	0.06 ± 0.02
Patients	99	47.0 ± 1.6 (18 to 79)	52/47	1.35 ± 0.08[b]	3.69 ± 0.22[c]
MCNS	18	39.7 ± 3.3 (18 to 63)	10/8	0.96 ± 0.09	4.89 ± 0.58
FGS	10	45.4 ± 6.1 (22 to 74)	6/4	1.41 ± 0.25	4.55 ± 0.56
IgA-N	30	41.2 ± 2.8 (19 to 67)	13/17	1.42 ± 0.13	2.09 ± 0.15[d,e]
MN	24	55.5 ± 2.4 (29 to 70)[f]	13/11	1.01 ± 0.08	3.8 ± 0.42
DM-N	17	54.1 ± 4.0 (24 to 79)	10/7	2.08 ± 0.26[g,h]	4.60 ± 0.53

[a] Serum creatinine value from DM-N patients was higher, whereas urinary protein excretion from IgA-N patients was lower than other proteinuric patients. S_{Cr}, serum creatinine; UP/U_{Cr}, urinary protein excretion; MCNS, minimal change nephrotic syndrome; FGS, focal glomerular sclerosis; IgA-N, IgA nephropathy; MN, membranous nephropathy; DM-N, diabetic nephropathy.
[b] $P < 0.05$ patients *versus* controls.
[c] $P < 0.01$ patients *versus* controls.
[d] $P < 0.01$ IgA-N *versus* MCNS and DM-N.
[e] $P < 0.05$ IgA-N *versus* MN and FGS.
[f] $P < 0.05$ MN *versus* MCNS and IgA-N.
[g] $P < 0.05$ DM-N *versus* IgA-N.
[h] $P < 0.01$ DM-N *versus* MCNS and MN.

concentration. Similarly, urinary protein excretion rate was calculated by the ratio of urinary protein concentration to the urinary creatinine concentration.

Administration of Oral Sodium Bicarbonate

Eleven patients with proteinuria and moderate renal insufficiency (two with FGS, five with IgA-N, four with DM-N) were treated with 3.5 g/d of oral sodium bicarbonate. All of these patients showed mild-to-moderate metabolic acidosis by arterial blood gas analysis (pH $<$ 7.35, base excess <-2 mEq/L, HCO_3^- $<$22 mEq/L), and moderate renal insufficiency (serum creatinine level $>$2.0 mg/dl). Data of these patients are given in Table 2. CAP in the urine and plasma were measured before and 14 d after sodium bicarbonate administration. Blood gas analysis and measurement of serum creatinine level and urinary protein excretion were performed simultaneously.

Statistical Analyses

All values are expressed as mean ± SEM. Statistical analysis for comparison among groups of patients with various diseases was performed by one-factor ANOVA. Further analysis between two groups was performed using the Scheffé *F* test. For evaluation of the effect of sodium bicarbonate therapy on the urinary excretion of CAP, the Wilcoxon test was used. Correlation coefficients (r_s) were calculated by Spearman analysis.

Results

Western Blot Analysis

The urine from MCNS patients did not show reaction bands for C3, C3b, or iC3b (Figure 1, lane H), whereas urine samples from proteinuric patients other than MCNS showed the reaction bands (Figure 1 lanes I through L). The bands for iC3b as well as for C3b were strongly detected in patients with DM-N and FGS. Thus, urinary excretion of CAP was different in the individual patient even when there was significant proteinuria (Figure 1).

Comparison of Urinary Excretion of CAP between Healthy Subjects and Proteinuric Patients

CAP (iC3b, Bb, and MAC) were almost undetectable or at the baseline level in the urine of healthy subjects (Table 3). There was significant increase of urinary CAP excretion in the urine of proteinuric patients as a whole.

Urinary Excretion of CAP According to Renal Histology

Urinary excretion of CAP at C3 level (iC3b and Bb) was significantly higher in FGS and DM-N. Urinary MAC excretion was similarly increased in FGS and DM-N, but it was also increased in MN patients (Table 3). Thus, the urine of MN patients revealed isolated increases of MAC. In MCNS, urinary excretion of CAP was comparable to that of healthy subjects. Thus, the subsequent analysis was done in the proteinuric patients excluding MCNS.

Table 2. Comparison of clinical parameters and urinary excretion of CAP before and after sodium bicarbonate administration[a]

Treatment by Sodium Bicarbonate	S_{Cr} (mg/dl)	UP/U_{Cr}	HCO_3^- (mEq/L)	BE (mEq/L)	P-iC3b (μg/ml)	P-Bb (μg/ml)	P-MAC (ng/ml)	U-iC3b/U_{Cr}	U-Bb/U_{Cr}	U-MAC/U_{Cr}
Before (n = 11)	4.32 ± 0.41	2.32 ± 0.40	16.4 ± 0.66	−8.51 ± 0.63	27.50 ± 7.36	0.55 ± 0.07	172.59 ± 38.71	22.80 ± 9.50	1.05 ± 0.31	315.34 ± 98.04
After (n = 11)	4.39 ± 0.48	1.96 ± 0.38	21.33 ± 0.66[b]	−2.44 ± 0.64[b]	24.90 ± 6.52	0.56 ± 0.09	186.23 ± 44.93	12.95 ± 4.31[c]	0.78 ± 0.26[c]	199.82 ± 73.34[b]

[a] CAP, complement activation products; BE, base excess; P, plasma; MAC, membrane attack complex. Other abbreviations as in Table 1.
[b] $P < 0.01$ before *versus* after (by Wilcoxon test).
[c] $P < 0.05$ before *versus* after (by Wilcoxon test).

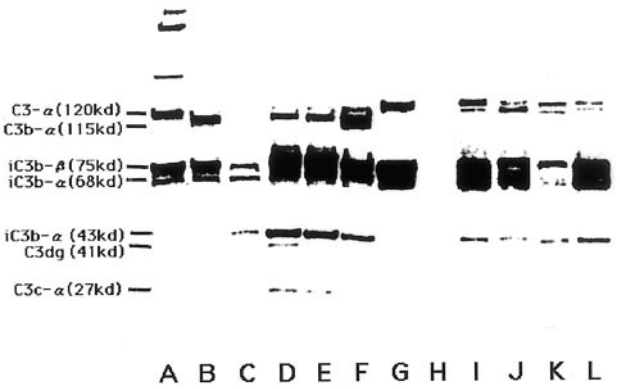

Figure 1. Western blot analysis of urinary C3 fragment under reduced conditions. Lane A, native C3; lane B, C3b; lane C, iC3b; lane D, C3b + soluble complement receptor type 1 (sCR1) 80 μg/ml + factor I; lane E, C3b + sCR1 8 μg/ml + factor I; lane F, C3b + sCR1 0.08 μg/ml + factor I; lane G, normal plasma; lane H, urine from minimal change nephrotic syndrome (MCNS) patient; lane I, urine from diabetic nephropathy patient; lane J, urine from IgA nephropathy patient; lane K, urine from membranous nephropathy patient; lane L, urine from focal glomerular sclerosis patient. In lanes D through F, C3c-α (27 kD) and C3dg (41 kD) are shown as degradation products of iC3b-α (68 kD) by factor I and sCR1 80 μg/ml. The urine from MCNS patients did not show reaction bands for C3, C3b, or iC3b, whereas urine samples from proteinuric patients other than MCNS showed the reaction bands for iC3b as well as for C3b.

Correlation between Urinary Excretion of CAP, Clinical Parameters, and Plasma Levels of CAP

Urinary excretion levels of iC3b, Bb, or MAC were significantly correlated with each other (Table 4). However, there was no significant correlation between plasma level and urinary excretion of CAP. Urinary excretion of CAP was significantly correlated with urinary protein excretion rate. Urinary excretion of iC3b and Bb was inversely correlated with the reciprocal of the serum creatinine ($1/S_{Cr}$), whereas urinary excretion of MAC was not correlated with renal function. However, when MN was excluded, urinary MAC excretion was inversely correlated with renal function.

Urinary Excretion of CAP According to The Level of Proteinuria

Urinary protein excretion (UP/U-Cr) was graded into five levels: <1.74 per day, 1.75 to 3.49 per day, 3.50 to 5.24 per day, 5.25 to 6.99 per day, and >7.0 per day. Urinary excretion of CAP showed marked increases when urinary protein excretion exceeded 3.5 per day (nephrotic range) (Figure 2).

Effect of Sodium Bicarbonate Therapy on Urinary Excretion of CAP

Urinary excretion levels of CAP were significantly reduced 2 wk after the oral administration of sodium bicarbonate. The effect of sodium bicarbonate seemed more evident in patients who showed a higher level of urinary CAP excretion (Figure 3). Metabolic acidosis as assessed by bicarbonate ion level and base excess was significantly improved by the sodium bicarbonate administration, whereas the other parameters such as serum creatinine level, urinary protein excretion, and plasma level of CAP were not affected by this treatment (Table 2).

Discussion

The hypothesis that complement is activated in the urine of patients with proteinuria (20,24,28,38) was tested in the present work. There were three new findings.

First, urinary excretion levels of CAP were elevated in the proteinuric patients excluding MCNS, and urinary excretion of CAP was significantly correlated with degree of urinary protein excretion and renal function. In MCNS patients, selectivity of glomerular barrier for plasma proteins was reported to be high (41). Therefore, the complement proteins such as C3 (molecular weight, approximately 200 kD) cannot be filtered into the urinary space. Indeed, C3 and related molecules were not detected at all by Western blot in the urine of MCNS patients. Except for MCNS, it was suggested by the present study that the amount of complement proteins was proportionate to the degree of proteinuria. Because the urinary excretion rate of CAP was markedly increased when the degree of proteinuria exceeded nephrotic range, the complement activation process might be accelerated in the nephrotic condition. It was believed that the optimal condition for the efficient complement activation such as density of complement molecules was fulfilled in the nephrotic condition. Rustom and colleagues reported that ammonium synthesis in the proximal tubules was increased in the proteinuric condition (37). Therefore, it is also probable that the increased synthesis of ammonium, an activator of alternative pathways of complement, was ample enough in the proximal tubules to activate complement under the nephrotic condition. Similarly, in the condition of renal insufficiency, ammonium production in the remnant kidney was reported to be increased (34). Therefore, complement activation was accelerated by the increased concentration of ammonium in the remnant nephron (35). In addition, there has been a report that functioning Factor D, a necessary enzyme that promotes C3 activation cycle by cleaving Factor B into Ba and Bb, is significantly increased in the urine of patients with chronic renal failure (42). All of these data suggest that complement activation is accelerated in the patients with reduced renal function.

Second, the urinary excretion rate of CAP was different according to the type of glomerular injury. FGS and DM-N showed markedly elevated excretion of CAP. This finding was originally reported by Ogrodowski *et al.* (28). Specifically, DM-N showed the largest excretion of CAP into urine. This is due to the presence of high levels of proteinuria and renal insufficiency (Table 1). In contrast, IgA-N patients excreted much less CAP into urine. This might be due to the limited degree of proteinuria in IgA-N patients. One of the interesting findings in the present study is the fact that, in MN, the urinary excretion rate of CAP at C3 level (iC3b and Bb) and that of MAC was dissociated. The finding that there was isolated elevation of urinary MAC excretion but not iC3b or Bb suggested that the origin of MAC was glomerulus by the mechanisms of shedding (27), and the activation of alternative path-

Table 3. Comparison of urinary excretion of CAP between healthy subjects and proteinuric patients[a]

Group	U-iC3b/U_{Cr}	U-Bb/U_{Cr}	U-MAC/U_{Cr}	P-iC3b (μg/ml)	P-Bb (μg/ml)	P-MAC (ng/ml)
Control subjects	0.09 ± 0.03	0.10 ± 0.03	23.24 ± 5.46	81.47 ± 18.01	0.59 ± 0.14	109.45 ± 36.60
Patients	18.75 ± 3.89[b]	0.83 ± 0.12[b]	288.37 ± 36.36[b]	166.26 ± 25.94	0.76 ± 0.04	199.93 ± 15.40
MCNS	0.52 ± 0.10	0.13 ± 0.03	23.63 ± 3.19	78.34 ± 28.47	0.69 ± 0.08	148.42 ± 20.84
FGS	47.00 ± 18.86[c]	1.46 ± 0.27[d]	362.10 ± 64.82[e]	176.87 ± 57.78	0.82 ± 0.11	172.06 ± 20.60
IgA-N	6.76 ± 2.42	0.39 ± 0.05	117.54 ± 14.98	148.74 ± 39.87	0.78 ± 0.10	228.27 ± 41.04
MN	6.68 ± 2.03	0.52 ± 0.14	372.64 ± 72.54[f,g]	263.59 ± 58.86	0.81 ± 0.10	215.70 ± 19.22
DM-N	59.66 ± 14.45[h]	2.39 ± 0.46[h]	707.84 ± 121.09[i,j]	146.64 ± 90.70	0.71 ± 0.10	198.58 ± 37.18

[a] Abbreviations as in Tables 1 and 2.
[b] $P < 0.05$ patients *versus* controls.
[c] $P < 0.05$ FGS *versus* MCNS, IgA-N, and MN.
[d] $P < 0.05$ FGS *versus* MCNS and IgA-N.
[e] $P < 0.05$ FGS *versus* MCNS.
[f] $P < 0.01$ MN *versus* MCNS.
[g] $P < 0.05$ MN *versus* IgA-N.
[h] $P < 0.01$ DM-N *versus* MCNS, IgA-N, and MN.
[i] $P < 0.01$ DM-N *versus* MCNS and IgA-N.
[j] $P < 0.05$ DM-N *versus* MN.

Table 4. Correlation between urinary excretion of CAP, clinical parameters, and plasma levels of CAP[a]

	1/S_{Cr}	UP/U_{Cr}	U-iC3b/U_{Cr}	U-Bb/U_{Cr}	U-MAC/U_{Cr}	P-iC3b	P-Bb	P-MAC
1/S_{Cr}		−0.005	−0.467[b]	−0.303[c]	−0.048 (−0.274[c])[d]	0.04	−0.172	0.107
UP/U_{Cr}			0.546[b]	0.478[b]	0.598[b]	−0.03	−0.01	−0.068
U-iC3b/U_{Cr}				0.676[b]	0.473[b]	−0.108	−0.055	−0.13
U-Bb/U_{Cr}					0.508[b]	−0.124	−0.069	−0.101
U-MAC/U_{Cr}						−0.131	−0.004	0.094
P-iC3b							0.444[b]	0.41[b]
P-Bb								0.32[b]
P-MAC								

[a] This analysis was performed in the proteinuric patients excluding MCNS ($n = 81$). Correlation coefficients (r_s) are expressed in figures. Urinary excretion levels of iC3b, Bb, or MAC were significantly correlated with each other. There was no significant correlation between plasma level and urinary excretion of CAP. Urinary CAP was significantly correlated with urinary protein excretion rate. Urinary iC3b and Bb was inversely correlated with reversed serum creatinine (1/S_{Cr}), whereas urinary excretion of U-MAC was not correlated with renal function. However, when MN was excluded, urinary MAC excretion was significantly correlated with 1/S_{Cr}. Abbreviations as in Tables 1 and 2.
[b] $P < 0.001$.
[c] $P < 0.01$.
[d] MN was excluded.

ways of complement in the urinary space was not amply induced. It still remains to be elucidated whether isolated urinary excretion of MAC is due to relatively higher selectivity of glomerular filtration barrier for the plasma proteins compared to FGS and DM-N, or whether it is due to the preserved renal function. Because patients with MN and IgA-N have a better prognosis (less frequency of renal death) than those with FGS and DM-N, the present data are in favor of the hypothesis of complement-mediated tubular injury. Thus, CAP at C3 level can be the better marker of the complement activation in the tubules.

Third, urinary excretion of CAP in patients with mild-to-moderate renal insufficiency and metabolic acidosis was significantly reduced by the correction of acidosis by oral sodium bicarbonate. Because it is well known that ammonium acts as a C3 activator, and ammonium production in the proximal tubular cells is markedly increased when there is metabolic acidosis, the present data are consistent with the notion that complement activation in the urinary space is accelerated under the condition of metabolic acidosis, and that correction of acidosis by alkali administration reduces complement activation in the urine of proteinuric patients (43). Although the serum creatinine level of the patients was not affected by oral sodium bicarbonate, it can be expected that the sustained correction of acidosis might delay the progression of renal injury.

The data obtained in the present work further demonstrated

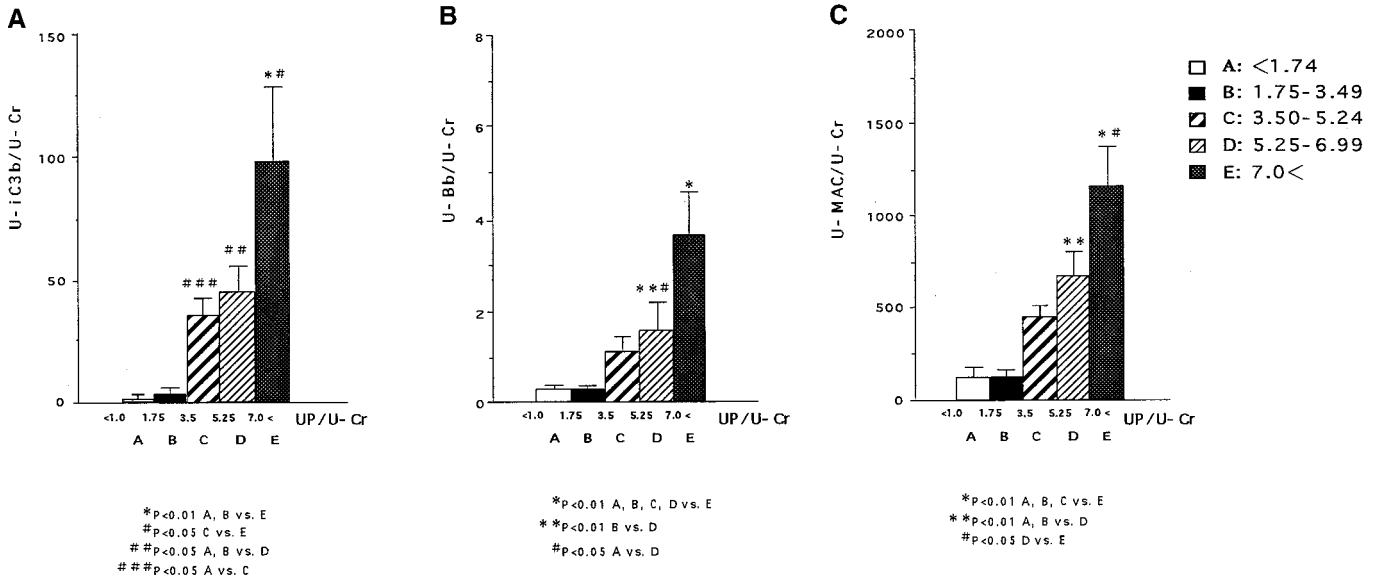

Figure 2. Urinary excretion of complement activation products (CAP) according to the level of proteinuria. (A) U-iC3b. (B) U-Bb. (C) U-membrane attack complex (MAC). Urinary excretion of CAP showed a marked increase when urinary protein excretion exceeded 3.5 g/d (nephrotic range).

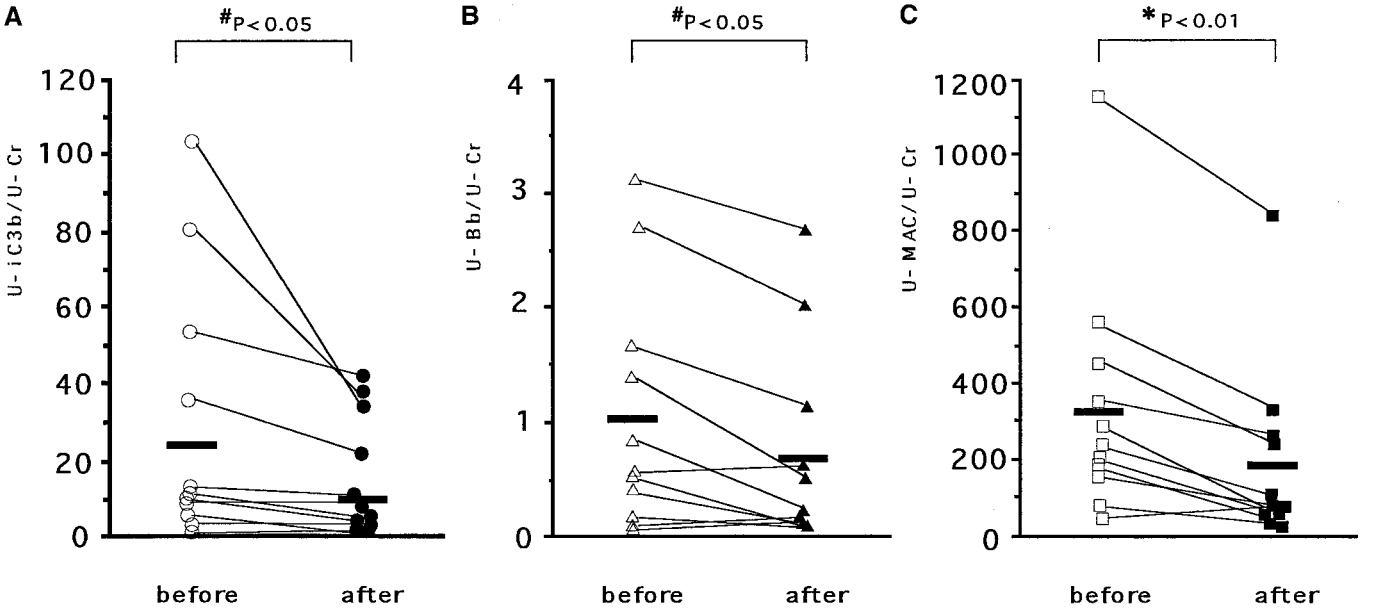

Figure 3. Effect of sodium bicarbonate therapy on urinary excretion of CAP. Urinary excretion levels of CAP were significantly reduced 2 wk after the oral administration of sodium bicarbonate.

the importance of complement proteins filtered into the tubular lumen in the proteinuric condition. The factors concerning the activation of complement in the tubules, such as degree of proteinuria, renal function, and acidosis, are also demonstrated to be important for the complement activation in the proteinuric patients. A study to elucidate whether the patients with increased urinary excretion of CAP show faster decline of renal function is now under investigation.

Acknowledgments

This work was supported in part by a 1998 Research Grant from the Aichi Kidney Foundation and a grant from Scientific Reset Expenses for Health and Welfare Programs, Funds for Comprehensive Research on Long Term Chronic Disease (Renal Failure). We thank the doctors in the associated hospitals for providing the urine and plasma samples. The excellent technical assistance of N. Suzuki, N. Asano, T. Kataoka, and Y. Fujitani is greatly appreciated.

References

1. Schainuck LI, Striker GE, Cutler RE, Benditt EP: Structural-functional correlations in renal disease. Part II. The correlations. *Hum Pathol* 1: 631–640, 1970
2. Bohle A, Wehrmann M, Bogenschutz O, Batz C, Vogl W, Schmitt H, Muller CA, Muller GA: The long-term prognosis of

the primary glomerulonephritis: A morphological and clinical analysis of 1747 cases. *Pathol Res Pract* 188: 908–924, 1992
3. Peterson JC, Adler S, Burkart JM, Greene T, Hebert LA, Hunsicker L, King AJ, Klahr S, Massry SG, Seifter JL, for the MDRD Study Group: Blood pressure control, proteinuria and the progression of renal disease. *Ann Intern Med* 123: 754–762, 1995
4. Remuzzi G, Ruggenenti P, Benigni A: Understanding the nature of renal disease progression. *Kidney Int* 51: 2–15, 1997
5. Ruggenenti P, Perna A, Mosconi L, Pisoni R, Remuzzi G, on behalf of the "Gruppo Italiano di Studi Epidemiologici in Nefrologia" (GISEN): Urinary protein excretion rate is the best independent predictor of ESRF in non-diabetic proteinuric chronic nephropathies. *Kidney Int* 53: 1209–1216, 1998
6. Thomas ME, Schreiner GF: Contribution of proteinuria to progressive renal injury: Consequences of tubular uptake of fatty acid bearing albumin. *Am J Nephrol* 13: 385–398, 1993
7. Kees-Folts D, Sadow JL, Schreiner GF: Tubular catabolism of albumin is associated with the release of an inflammatory lipid. *Kidney Int* 45: 1697–1709, 1994
8. Zoja C, Donadelli R, Colleoni S, Figliuzzi M, Bonazzola S, Morigi M, Remuzzi G: Protein overload stimulates RANTES production by proximal tubular cells depending on NF-κB activation. *Kidney Int* 53: 1608–1615, 1998
9. Alfrey AC, Froment DH, Hammond WS: Role of iron in the tubulo-interstitial injury in nephrotoxic serum nephritis. *Kidney Int* 36: 753–759, 1989
10. Chen L, Wang Y, Tay YC, Harris DCH: Proteinuria and tubulointerstitial injury. *Kidney Int* 52: S60–S62, 1997
11. Ong ACM, Jowett TP, Moorhead JF, Owen JS: Human high density lipoproteins stimulate endothelin-1 release by cultured human renal proximal tubular cells. *Kidney Int* 46: 1315–1321, 1994
12. Nath KA: Tubulointerstitial changes as a major determinant in the progression of renal damage. *Am J Kidney Dis* 20: 1–17, 1992
13. Eddy AA: Experimental insights into the tubulointerstitial disease accompanying primary glomerular lesions. *J Am Soc Nephrol* 5: 1273–1287, 1994
14. Benigni A, Zoya C, Remuzzi G: The renal toxicity of sustained glomerular protein traffic. *Lab Invest* 73: 461–468, 1995
15. Burton C, Harris KPG: The role of proteinuria in the progression of chronic renal failure. *Am J Kidney Dis* 27: 765–775, 1996
16. Bruzzi I, Benigni A, Remuzzi G: Role of increased glomerular protein traffic in the progression of renal failure. *Kidney Int* 52[Suppl 62]: S29–S31, 1997
17. Sato K, Ullrich KJ: Serum-induced inhibition of isotonic fluid absorption by the kidney proximal tubule. II. Evidence that complement is involved. *Biochem Biophys Acta* 354: 182–187, 1974
18. Camussi G, Rotunno M, Segoloni G, Brentjens JR, Andres GA: In vitro alternative pathway activation of complement by the brush border of proximal tubules of normal rat kidney. *J Immunol* 128: 1659–1663, 1982
19. Biancone L, David S, Pietra DV, Montrucchio G, Cambi V, Camussi G: Alternative pathway activation of complement by cultured human proximal tubular epithelial cells. *Kidney Int* 45: 451–460, 1994
20. Williams DJ, Coles AG: Proteinuria: A direct cause of renal morbidity? *Kidney Int* 45: 443–450, 1994
21. Khan TN, Sinniah R: Role of complement in renal tubular damage. *Histopathology* 26: 351–356, 1995
22. David S, Biancone L, Caserta C, Bussolati B, Cambi V, Camussi G: Alternative pathway complement activation induces proinflammatory activity in human proximal tubular epithelial cells. *Nephrol Dial Transplant* 12: 51–56, 1997
23. Abbate M, Zoya C, Rottoli D, Corna D, Perico N, Bertani T, Remuzzi G: Antiproteinuric therapy while preventing the abnormal protein traffic in proximal tubule abrogates protein-and complement-dependent interstitial inflammation in experimental renal disease. *J Am Soc Nephrol* 10: 804–813, 1999
24. Camussi G, Stratta P, Mazzucco G, Gaido M, Tetta C, Castello R, Rotunno M, Vercellone A: In vivo localization of C3 on the brush border of proximal tubules of kidneys from nephrotic patients. *Clin Nephrol* 23: 134–141, 1985
25. Schulze M, Baker PJ, Perkinson DT, Johnson RJ, Ochi RF, Stahl RAK, Couser WG: Increased urinary excretion of C5b-9 distinguishes passive Heymann nephritis in the rat. *Kidney Int* 35: 60–68, 1989
26. Schulze M, Donadio JV, Pruchno CJ, Baker PJ, Johnson RJ, Stahl RAK, Watkins S, Martin DC, Wurzner R, Gotze O, Couser WG: Elevated urinary excretion of the C5b-9 complex in membranous nephropathy. *Kidney Int* 40: 533–538, 1991
27. Kerjaschki D, Schulze M, Binder S, Kain R, Ojha PP, Susani M, Horvat R, Baker PJ, Couser WG: Transcellular transport and membrane insertion of the C5b-9 membrane attack complex of complement by glomerular epithelial cells in experimental membranous nephropathy. *J Immunol* 143: 546–552, 1989
28. Ogrodowski JL, Hebert LA, Sedmak D, Cosio FG, Tamerius J, Kolb W: Measurement of SC5b-9 in urine patients with the nephrotic syndrome. *Kidney Int* 40: 1141–1147, 1991
29. Morita Y, Nomura A, Yuzawa Y, Nishikawa K, Hotta N, Shimizu F, Matsuo S: The role of complement in the pathogenesis of tubulointerstitial lesions in rat mesangial proliferative glomerulonephritis. *J Am Soc Nephrol* 8: 1363–1372, 1997
30. Nomura A, Morita Y, Maruyama S, Hotta N, Nadai M, Wang L, Hasegawa T, Matsuo S: Role of complement in acute tubulointerstitial injury of rats with aminonucleoside nephrosis. *Am J Pathol* 151: 539–547, 1997
31. Ichida S, Yuzawa Y, Okada H, Yoshioka K, Matsuo S: Localization of the complement regulatory proteins in the normal human kidney. *Kidney Int* 46: 89–96, 1994
32. Tack BF, Harrison RA, Janatova J, Thomas ML, Prahl JW: Evidence for presence of an internal thiolester bond in the third component of human complement. *Proc Natl Acad Sci USA* 77: 5764–5788, 1980
33. Howard JB: Methylamine reaction and denaturation-dependent fragmentation of complement component 3. *J Biol Chem* 255: 7082–7084, 1980
34. Schoolwerth AC, Sandler RS, Hoffman PM, Klahr S: Effects of nephron reduction and dietary protein content on renal ammoniagenesis in the rat. *Kidney Int* 7: 397–404, 1975
35. Nath KA, Hostetter MK, Hostetter TH: Pathophysiology of chronic tubulo-interstitial disease in rats: Interactions of dietary acid load, ammonia, and complement component C3. *J Clin Invest* 76: 667–675, 1985
36. Tolins JP, Hostetter MK, Hostetter TH: Hypokalemic nephropathy in the rat: Role of ammonia in chronic tubular injury. *J Clin Invest* 79: 1447–1458, 1987
37. Rustom R, Jackson MJ, Critchley M, Bone JM: Tubular metabolism of aprotinin 99mTc and urinary ammonia: Effects of proteinuria. *Miner Electrolyte Metab* 18: 108–112, 1992

38. Matsuo S, Morita Y, Mizuno M, Nishikawa K, Yuzawa Y: Proteinuria and damage to tubular cells: Is complement a culprit? *Nephrol Dial Transplant* 13: 2723–2726, 1998
39. Rustom R, Maltby P, Grime JS, Hughes A, Costigan M, Shenkin A, Critchley M, Bone JM: Tubular peptide hypermetabolism and urinary ammonia in chronic renal failure in man: A maladaptive response? *Nephron* 79: 306–311, 1998
40. Weisman HF, Bartow T, Leppo MK, Marsh JHC, Carson GR, Concino MF, Boyle MP, Roux KH, Weisfeldt ML, Fearon DT: Soluble human complement receptor type 1: In vivo inhibitor of complement suppressing post-ischemic myocardial inflammation and necrosis. *Science* 249: 146–151, 1990
41. Nadasdy T, Silva FG, Hogg RJ: Minimal change nephrotic syndrome-focal sclerosis complex (including IgM nephropathy and diffuse mesangial hypercellularity). In: *Renal Pathology with Clinical and Functional Correlations*, edited by Tisher CC, Brenner BM, Philadelphia, Lippincott, 1994, pp 330–389
42. Volanakis JE, Barnum SR, Guddens M, Galla JH: Renal filtration and catabolism of complement protein D. *N Engl J Med* 312: 395–399, 1985
43. Rustom R, Grime JS, Costigan M, Maltby P, Hughes A, Taylor W, Shenkin A, Critchley M, Bone JM: Oral sodium bicarbonate reduces proximal renal tubular peptide catabolism, ammoniogenesis, and tubular damage in renal patients. *Ren Failure* 20: 371–382, 1998