Inhibitory Effect of Free Sialic Acid on Complement Activation and its Significance in Hypocomplementemic Glomerulonephritis

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The role of free sialic acid on complement activation was investigated. The serum levels of free sialic acid and total sialic acid were measured by previously described methods in 16 patients with acute post-infectious glomerulonephritis (AGN), 27 patients with systemic lupus erythematosus (SLE), 15 patients with persistent hypocomplementemic membranoproliferative glomerulonephritis (MPGN), and 13 healthy controls. A statistical study demonstrated an increased level of free sialic acid in patients with AGN and SLE in which the hypocomplementemia improved throughout the course and a decreased level of free sialic acid in patients with MPGN and SLE in which hypocomplementemia continued throughout the course. The levels of total sialic acid were significantly increased in patients with AGN and SLE and were significantly decreased in patients with MPGN. There was no correlation between the levels of free sialic acid and total sialic acid in patients with AGN, in whom the levels of both total and free sialic acids were increased. To examine the effect of free sialic acid on the complement cascade, lipopolysaccharide (LPS) was incubated with normal human serum (NHS) in the various concentrations of N-acetyl neuraminic acid (NANA), a member of the sialic acid group. The incubation mixtures were examined by enzyme immunoassay using monoclonal anti-*i*C3b antibody or anti-Bb antibody. Native C3 or Factor B in NHS broke down less following the addition of NANA. To elucidate the role of NANA on the hemolytic function of C3, a rabbit erythrocyte (Ra E) hemolytic assay was carried out. Ra E lysed completely in the presence of R3 with native C3. However, hemolysis occurred to a lesser degree in C3-depleted serum (R3) or R3 with NANA-treated C3. To investigate the influence of NANA on complement components, the levels of complement components were measured in the incubation mixture with various doses of NANA and NHS. The levels of C3 and C5 were significantly decreased after the addition of NANA, even though the levels of Factor H and Factor I were not markedly changed. These data indicate that NANA exerts an influence on the complement components even though it has no effect on the regulatory proteins of complement. Our in vitro findings, together with the in vivo data, suggest that free sialic acid might have an inhibitory effect on the activation of C3 and the following complement cascade, and might also have been responsible for the improvement of hypocomplementemia. J. Clin. Lab. Anal. 13:173–179, 1999. © 1999 Wiley-Liss, Inc.

Key words: sialic acid; hypocomplementemia; acute post-infectious glomerulonephritis; membranoproliferative glomerulonephritis; systemic lupus erythematosus

INTRODUCTION

Persistent hypocomplementemia often occurs in patients with lupus nephritis and membranoproliferative glomerulonephritis (MPGN) (1,2). Continuous activation of the complement pathway is said to be implicated in the pathogenesis of chronic glomerular injury (3,4). Previous reports have suggested that circulating factors (e.g., immune complex, C3 nephritic factor) may be involved in the activation of the complement pathway (5,6). However, we have experienced patients with lupus nephritis in remission with the continuous detection of autoantibody and patients with continuous hypocomplementemia without the detection of C3 nephritic factor, autoantibody to C3bBb. Prolonged hypocomplementemia in chronic glomerular disease is governed by multiple mechanisms. Nevertheless, it is necessary to prevent

continuous complement activation and prolonged tissue injury as a self-defense mechanism.

Sialic acids are components of serum glycoproteins and glycolipids on cell surfaces (7,8). They form antigenic determinants as terminal components of glycoconjugates, while they often mask antigenic sites in other cases. A relative lack of sialic acid in glycoconjugates has been found to be important for complement activation (9). Pangburn and Müller-Eberhard reported that restriction of Factor H control could be induced on the surface of certain cells by removal of the cell-surface sialic acid with neuraminidase (10). The glyco-protein layer, rich in sialic acids, is said to act as an immunobarrier. Suppression of antigenicity by sialic acid appears to be a general feature of molecules and cells. Partial loss of these sugars has been proposed as one cause of autoimmune disease (11). Such masking of specific recognition sites on molecules and cells is the most fascinating and possibly the most important role of sialic acids (12). However, the precise role of free sialic acid in complement activation in the fluid phase has not yet been clearly established.

In the present study, the effects of free sialic acid on complement components and the activating pathway of complement were investigated in vitro. Also, serum levels of free sialic acid in sera of patients with transient and persistent hypocomplementemia were determined. The purposes of our study were to elucidate the effect of free sialic acid in complement activation and to examine the mechanisms underlying persistent hypocomplementemia in chronic glomerular injury.

MATERIALS AND METHODS

Chemicals and Reagents

Crystalline NANA, ethylenediaminetetraacetic acid (EDTA), and ethyleneglycol-bis(b-aminoethylether)N,N′-tetraacetic acid (EGTA) were purchased from Nakarai Tesque (Kyoto, Japan), and lipopolysaccharide (LPS) from Escherichia coli was purchased from Sigma Chemical (St. Louis, MO). Native C3 was isolated by the method described previously (13). Purified C3 was checked for monospecificity and biological activity. The antisera against human C3, C5, and Factor B were purchased from Behringwerke (Marburg, Germany); anti-Properdin and anti-Factor I from ICN Immunobiologicals (Lisle, IL); and anti-Factor H from Miles Scientific (Naperville, IL).

Serum Samples

Normal human serum (NHS) for the incubation and 13 sera as controls were obtained from healthy donors. Serum samples were obtained from 16 patients with post-infectious acute glomerulonephritis (AGN) with C3 depression, 18 patients with lupus nephritis (SLE), and 15 patients with MPGN who were persistently hypocomplementemic (C3 < 50 mg/dL); in addition, sera were obtained from 9 of the 18 patients with SLE who had become normocomplementemic (C3 > 50 mg/dL) after treatment. All the samples and sera from healthy controls were assayed for total and free sialic acid measurements.

Estimation of Complement Component Levels

The levels of complement components (C3, C5, Factor B, Factor H, Factor I, and Properdin) were determined by the single radial immunodiffusion method (SRID) in incubation mixtures of NANA and NHS (14). The data were expressed as a percentage of the levels of each component in NHS.

Quantitation of Complement Breakdown Products

Activation of C3 or Factor B was checked from the generation of iC3b or Bb fragment employing an enzyme immunoassay (15). The used monoclonal antibody reacts with the neoantigen of iC3b or Bb fragment, but does not react with native C3 or Factor B. The assay kit was purchased from Quidel (San Diego, CA). This enzyme immunoassay utilized: (1) a microassay plate coated with monoclonal antihuman iC3b or Bb; (2) an HRP-conjugated anti-iC3b or anti-Bb; and (3) a chromogenic substrate. This monoclonal antibody reacts only with iC3b or Bb, but does not react with native C3 or Factor B. In preparation for this assay, 90 μl of NHS and 10 μl of LPS (10 mg/ml) were incubated with 10 μl of various concentrations of NANA solution (final concentration: 0 mMol/L, 12.5 mMol/L, 25.0 mMol/L, 50.0 mMol/L, 100.0 mMol/L) at 37°C for 30 min. LPS is known to activate alternative complement pathway in its large amount. The same volume of 0.2 M EDTA solution was added to the incubation mixture and was subjected to the microplate.

Rabbit Erythrocyte Hemolytic Assay

Rabbit erythrocyte (Ra E) hemolytic assay was performed according to a previously described method, followed by specific immunoabsorption (16). Ra E was collected and washed 3 times in Veronal-buffered saline containing 3 mM MgCl$_2$, 10 mM EGTA and gelatin (Mg-EGTA-GVB), and was then resuspended to 2×10^8/mL in the same buffer. C3-depleted serum (R3) was prepared by the incubation of NHS and yeast as described previously (17). R3 prepared by this method retained considerable C3 activity. One volume of R3 was diluted with 4 volumes of Mg-EGTA-GVB. Fifty μL of the purified C3 (5,000 SFU/mL, 120 μg) was preincubated at 37°C for 10 min with the same volume of NANA (500 μg) or phosphate-buffered saline (PBS). One hundred μL of RaE suspension and 50 μL of diluted R3 with 50 μL of other reagents were incubated at 37°C for 30 min, with frequent shaking, as follows: (1) Ra E + R3 + C3 (5,000 SFU in PBS); (2) Ra E + R3 + NANA-C3 (in PBS); (3) Ra E + R3 + NANA (in PBS); and (4) Ra E + R3 (+ PBS). After the incubation, 1.7 mL of Veronal-buffered saline containing 10 mM EDTA and gelatin (EDTA-GVB) was added. The tubes were centrifuged at 600g for 10 min. The optical density of the supernatant was measured at 414 nm.

Measurement of Free Sialic Acid in Serum

The serum levels of free sialic acid were determined by fluorometric assay as described by Hammond and Papermaster (18). Essentially, serum samples were deproteinized with the same volume of 30% trichloroacetate and centrifuged at 2,000 rpm for 20 min. The supernatants in a volume of 120 μL were neutralized with 100 μl of 1N NaOH. Fifty μL of freshly prepared 25 mM periodic acid in 125 mM HCl was added, and the mixture was vortexed prior to incubation at 37°C for 30 min. Excess periodate was reduced with 40 μL of 2% sodium arsenite in 0.5 N HCl. Thiobarbituric acid, 0.4 mL, was added, and the tubes were heated in a boiling-water bath for 7.5 min. The tubes were immediately immersed in ice water and 0.6 mL of n-butanol containing 5% HCl was added. After vortexing and centrifugation at 1,000g for 10 min, the butanol layer was separated. Fluorometric measurements were made with a spectrofluorometer. The emission at 570 nm was monitored using an excitation wave length of 550 nm.

Measurement of Total Sialic Acid in Serum

The serum level of total sialic acid was determined by enzymatic assay (19). The reagents were purchased from Kyokuto Pharmaceutical Industrial Co. (Tokyo, Japan). The precise contents were not described in the accompanying manual. The reagent kit consists of three solutions. Reagent A contained neuraminidase and 4-aminoantipyrine. Reagent B contained NANA-aldolase, pyruvate oxidase, thiamine pyrophosphate, flavin adenine dinucleotide, and peroxidase. Reagents A and B were dissolved in 20 mM phosphate buffer, pH 7.4, containing 10 mM $MgCl_2$ and 3.8 mM p-chlorophenol. Reagent C contained EDTA, disodium phosphate, sodium citrate and Triton X-405 dissolved in deionized water (pH 9.0). Twenty μL of serum was mixed with 0.5 mL of Reagent A and 0.5 mL of Reagent B, and incubated for 20 min at 37°C. After the incubation, 1 mL of Reagent C was added and the mixture was subjected to colorimetry at 550 nm. The reaction process involved neuraminidase hydrolysis of glycoprotein, cleavage of sialic acid to pyruvate by NANA-aldolase, oxidation of the pyruvate by pyruvate oxidase to produce hydrogen peroxidase, and colorimetry of the hydrogen peroxidase by the peroxidase-p-chlorophenol-4-amino-antipyrine method.

Statistical Analysis

In each study, the measurement was carried out three times. The data were expressed as the mean value of each measurement. Statistical analysis was performed by unpaired and two-tailed Student's t-test.

RESULTS

Serum Levels of Total and Free Sialic Acid

The levels of free and total sialic acid were measured (by the assay procedures described in Materials and Methods) using the sera of 16 patients with AGN, 27 patients with SLE, 15 patients with MPGN and 13 healthy controls. The levels of total sialic acid were significantly increased in patients with AGN and SLE, and were decreased in MPGN. The levels of free sialic acid were low in patients with MPGN, elevated in those with AGN, and not significantly different in those with SLE (Table 1). Statistical studies indicated that there was no correlation between the levels of free sialic acid and total sialic acid in patients with AGN, in whom the levels of both total and free sialic acids were increased. These data suggest that the level of free sialic acid was increased when hypocomplementemia was occasional and the glomerular damage was improved or minimal, whereas it was decreased when hypocomplementemia was persistent and glomerular damage was prolonged.

In SLE patients, the range of free sialic acid levels was widely spread, and there was no significance with respect to the normal controls. However, comparison of the transient hypocomplementemic and persistent hypocomplementemic groups showed that the difference in their free sialic acid levels was statistically significant ($P < 0.01$). The levels of free sialic acid were increased in the transient hypocomplementemic group, where the levels of complement components were improved by therapy. The levels of total sialic acid were elevated in both groups (Table 1). These findings imply that the level of total sialic acid might reflect disease activity, and that the level of free sialic acid might suggest the prognosis

TABLE 1. Serum Levels of Free and Total Sialic Acid in Patients With Hypocomplementemic Nephritis of Various Origins[a]

		Free sialic acid		Total sailic acid	
	n	(μMol/L)	(% normal)	(μMol/L)	(% normal)
NHS	13	16.0 ± 2.3	(101.2 ± 14.5 %)	1710 ± 290	(99.5 ± 16.9 %)
AGN	16	19.1 ± 2.2[*]	(121.0 ± 14.4 %)	2317 ± 533[*]	(134.8 ± 31.0 %)
MPGN	15	10.8 ± 3.8[*]	(68.0 ± 24.0 %)	1414 ± 320*	(82.3 ± 18.6 %)
SLE					
Total	27	14.4 ± 4.4	(91.0 ± 28.0 %)	2434 ± 699[**]	(141.6 ± 40.7 %)
Persistent	18	13.9 ± 4.2	(88.0 ± 26.5 %)	2257 ± 746	(131.3 ± 43.4 %)
Transient	9	20.2 ± 2.7[***]	(127.7 ± 16.9 %)	2588 ± 373	(150.6 ± 21.7%)

[a]Values: M ± SD μMol/L (M ± SD % normal).
[*]$P < 0.05$ vs. NHS; [**]$P < 0.01$ vs. NHS; [***]$P < 0.01$ vs. persistent hypocomplementemic lupus nephritis.

for the glomerular injury. Especially, the increasing level of free sialic acid might play a role in the continuation of complement activation.

Inhibition of Complement Breakdown by NANA

The activation of C3 and Factor B was measured by enzyme immunoassay in incubation mixtures consisting of NHS with various concentrations of NANA and a large amount of LPS as an activator of the alternative complement pathway. Ten μl of LPS (10 mg/mL) was incubated with 90 μL of NHS at 37°C for 30 min in the various concentrations of NANA (0–100 nMol/L). The study was carried out three times, and the data were expressed as the mean values of each measurement. C3 in NHS was cleaved into breakdown products, *i*C3b, after incubation without NANA (*i*C3b: 51 ± 8 μg/mL), but significantly little cleavage took place in the presence of NANA (12.5 mMol/L: 8.0 ± 2.0* μg/mL, 25.0 mMol/L: 0.0 ± 11.0* μg/mL, 50.0 mMol/L: 0.0 ± 13.0* μg/mL, 100.0 mMol/L: 1.0 ± 13.0* μg/mL; *: significant vs. *i*C3b after incubation without NANA, $P < 0.001$) (Fig. 1A). Factor B in NHS was also cleaved into breakdown products, Bb, after incubation without NANA (Bb: 25.5 ± 0.9 μg/mL), but significantly little cleavage was noted in the presence of NANA (12.5 mMol/L: 25.8 ± 0.0 μ/mL, 25.0 mMol/L: 5.7 ± 1.2# μg/mL, 50.0 mMol/L: 3.6 ± 0.5# μg/ML, 100.0 mMol/L: 1.0 ± 4.5# μg/mL; #: significant vs. Bb after incubation without NANA, $P < 0.001$ (Fig. 1B). These data indicate that 25.0 mMol/L of NANA, which was 150% of the concentration in healthy subjects, inhibited C3 and Factor B cleavage via complement activation.

Diminished Hemolytic Activity of C3 With NANA

To elucidate the role of NANA on the hemolytic function of C3, Ra E hemolysis assay was performed. Ra E, suspended to 2×10^8/mL in a test tube, and lysed completely in the presence of a R3 with native C3 (Absorption at O.D.414: 0.479 ± 0.013). However, hemolysis occurred to a significantly lesser degree without native C3 (Absorption at O.D.414: 0.137 ± 0.012) or with NANA-treated C3 (Absorption at O.D.414: 0.126 ± 0.089, $P < 0.001$ vs. R3 with native C3, and not significant vs. R3 without native C3). The background hemolysis shown in the negative control represented the residual hemolytic activity of C3 in R3 (Absorption at O.D.414: 0.127 ± 0.011) because C3 in R3 was not completely depleted in the course of refinement (Fig. 2). Further hemolysis was confirmed after incubation with the addition of native C3 (data not shown). These data revealed that NANA-treated C3 had already lost hemolytic activity. The study was carried out three times, and the data were expressed as the mean values of each measurement.

Influence of NANA on Complement Components

To examine the influence of NANA on the complement cascade, various doses of NANA (100–200 μg) were incubated with 50 μL of NHS at 37°C for 10 min and the serum levels of complement components were determined by the SRID method after 24-h dialysis in PBS. The study was carried out three times, and the data were expressed as the mean values of each measurement. The antigen levels of C3 and C5 were significantly decreased after incubation with NANA, and those of Factor B and Properdin revealed mild declines. However, the levels of Factor H and Factor I were not markedly altered. These data indicate that NANA exerts an influence on the activating components of the complement cascade even though it has no effect on the regulatory proteins of the complement pathway (Fig. 3).

DISCUSSION

Sialic acids are components of serum glycoproteins and glycolipids on cell surfaces. They form antigenic determi-
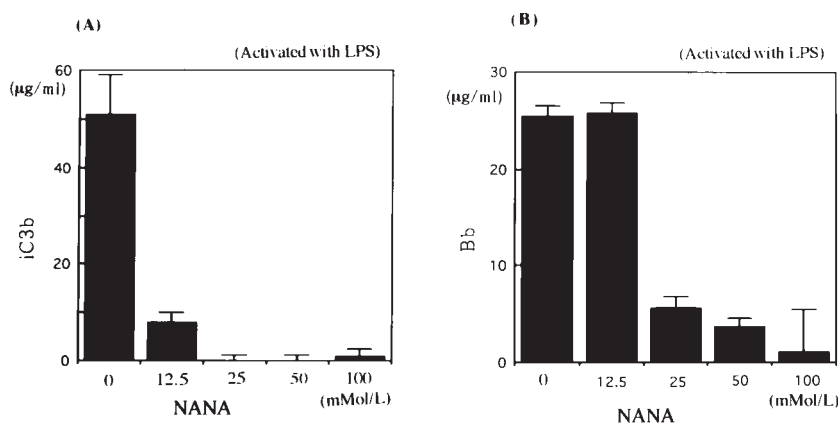

Fig. 1. Inhibition of C3 breakdown (A) and Factor B breakdown (B) by NANA. The activation of C3 and Factor B was estimated by enzyme immunoassay in incubation mixtures consisting of NHS with various concentrations of NANA and 10 mg/mL of LPS as an activator of the alternative complement pathway. C3 or Factor B in NHS was cleaved into the breakdown products after incubation without NANA, but was significantly little cleaved in the presence of NANA respectively. These data suggest that NANA might inhibit C3 and Factor B cleavage via complement activation.
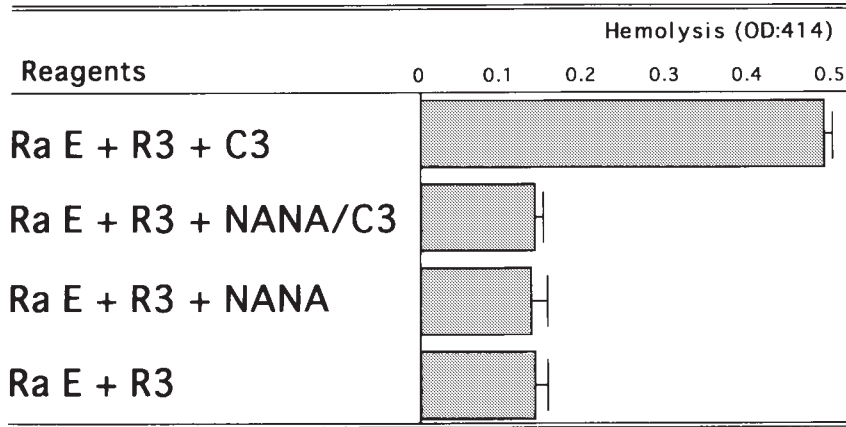
Fig. 2. Diminished hemolytic activity of C3 with NANA. One hundred µL of Ra E suspension (2×10^8/mL) and 50 µL of R3 with other reagents were incubated at 37°C for 30 min with frequent shaking. Ra E, suspended to 2×10^8/mL in a test tube, lysed completely in the presence of R3 with native C3. However, hemolysis occurred to a significantly lesser degree in the presence of R3 with NANA-treated C3 ($P < 0.001$ vs. R3 with native C3, not significant vs. R3 without native C3).

nants as terminal components of glycoconjugates, while they often mask antigenic sites in other cases. They are thus said to function as an immunobarrier (7,8). A relative lack of sialic acid in glycoconjugates has been found to be important for complement activation (9,10). In the present study, the inhibitory action of free sialic acid on complement activation was first examined. Our initial experiments revealed that the breakdown of C3 and Factor B was blocked by a member of the free sialic acid group, NANA. This phenomenon is reconfirmed by by C3 conversion study instead of *i*C3b generation, and by using Cobra venom factor instead of LPS

(data not shown). To elucidate the role of NANA on the hemolytic function of C3, we also examined the hemolytic activity of complement in the presence of NANA using Ra E and human complement components. The data revealed that NANA-treated C3 had no hemolytic activity and that further hemolysis was confirmed after the additional incubation with native C3. These findings indicate that free sialic acid might inhibit complement activation by masking the active binding site of complement components. However, it is said that there are no enzymatic binding sites on C3. Therefore, the following possibilities should be discussed. First, comple-
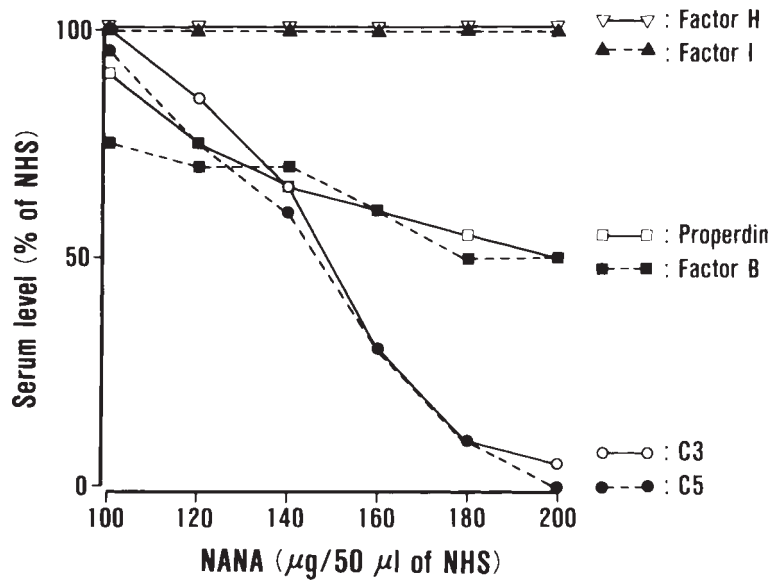

Fig. 3. Influence of NANA on complement components. Various doses of NANA (100–200 µg) was incubated with 50 µL of NHS at 37°C for 10 min, and the serum levels of complement components were estimated by the SRID method. The antigen levels of C3 and C5 were significantly decreased after the incubation with NANA, and those of Factor B and Properdin revealed mild declines. However, the levels of Factor H and Factor I were not markedly altered.

ment components might be denatured by acidification. Secondly, free sialic acid might nonenzymatically bind to C3. To investigate the role of NANA on the other components of complement, the levels of complement components were estimated by the SRID method in the incubation mixture with NHS and NANA. The serum levels of C3 and C5 were significantly decreased after the incubation with NANA; however, the levels of Factor H and Factor I were not markedly altered. These data indicate that NANA exerts an influence on the activating components of the complement cascade even though no effect on the regulatory proteins of the complement pathway was indicated. The phenomenon of complement components being more sensitive than the regulatory proteins of complement is thought to explain the mechanism for the inhibition of complement activation by free sialic acid.

Hypocomplementemia often occurs in patients with glomerular diseases (1,2). Complement activation has been said to be implicated in the pathogenesis of glomerular injury (3,4). It is important therefore to know whether the hypocomplementemia is occasional or persistent. Our study revealed that the free sialic acid levels were low in patients with persistent hypocomplementemia. In view of the fact that free sialic acid inhibits complement activation and that the glomerular damage tends to be minimal or improved in the occasional hypocomplementemic group, free sialic acid may act to prevent prolonged complement activation and subsequent glomerular injury by the complement system. These clinical data, together with the preliminary findings, suggest that free sialic acid might play some role in the improvement of hypocomplementemia. Our data indicated that 25.0 mMol/L of NANA, which is 150% greater than that of the concentration in NHS, inhibited C3 and Factor B cleavage via complement activation. The maximum level of free sialic acid among patients studied was 200% greater than that of NHS. This may be sufficient to regulate complement activation. Therefore, the level of free sialic acid independently reflects the improvement of hypocomplementemia. The serum level of total sialic acid reflects the level of acute phase reactants and is said to be a marker of inflammation (20,21). In our study, the level of total sialic acid was significantly higher than normal in patients with AGN and SLE. However, there was no correlation between the levels of free sialic acid and total sialic acid. It appears that the serum level of total sialic acid reflects disease activity, while the level of free sialic acid indicates the prognosis of the glomerular injury. In MPGN patients, the total sialic acid level was low even though the disease was in an active stage. Most of these patients exhibited severe nephrotic syndrome in the active stage, and their serum glycoproteins containing sialic acid were lost through the heavy proteinuria. In such cases, the total sialic acid level is not a suitable marker of disease activity. However, our present data showed that the level of free sialic acid independently reflects the improvement of hypocomplementemia. Therefore, the determination of free

sialic acid in serum is of value for following hypocomplementemic glomerulonephritis.

In summary, sialic acid in glycoconjugates has been found to be important for complement activation (8,10). However, the precise role of sialic acid in the fluid phase has not yet been clearly established. The present study indicates that free sialic acid might have an inhibitory effect on the activation of C3 and the subsequent complement pathway in vitro, and might play a role in the improvement of hypocomplementemia in vivo.

REFERENCES

1. Leibowitch L, Halwachs M, Wattel S. Glomerulonephritis and hypocomplementemia: pathophysiology and pathogenic implications. In: Hamburger J, Crosnier J, Grünfeld J, Maxwell MH, editors. Advances in Nephrology, vol. 9. Chicago: Year Book Medical Publishers; 1980. p 295–314.
2. Ohi H. The role of complement in membrano-proliferative glomerulonephritis (MPGN). Jpn J Nephrol 1987;20:1125–1139.
3. Glassock RJ. Clinical features of immunologic glomerular disease. In: Wilson CB, editor. Immunologic Mechanisms of Renal Disease. London: Churchill Livingstone; 1979. p 165–179.
4. Fujita T. Clinical study of complement breakdown products in patients with hypocomplementemic glomerulonephritis. Jpn J Nephrol 1982;24:845–853.
5. Seki M, Ohi H, Kojima H, Fujita T, Hatano M. A case of Hypocomplementemic mesangiocapillary glomerulonephritis: appearance of C3 NeF and another factor. Jpn J Nephrol 1987;29:585–588.
6. Fujita T, Ohi H, Seki M, Hatano M. Clinical evaluation and characterization of a unique C3 breakdown factor detected in a patient with acute glomerulonephritis. Nephron 1987;47:56–61.
7. Schauer R. Chemistry, metabolism, and biological functions of sialic acids. In: Tipson RS, Horton D, editors. Advances in Carbohydrate Chemistry and Biochemistry, vol. 40. London: Academic Press; 1982. p 131–234.
8. Shauer R. Sialic acids and their role as biological masks. In: Hall J, editor. Trends in Biochemical Science, vol. 10. Amsterdam: Elsevier Science Publishers; 1985. p 357–360.
9. Ourth DD, Bachinski LM. Bacterial sialic acid modulates activation of the alternative complement pathway of channel catfish. Develop Comp Immunol 1987;11:551–564.
10. Pangburn MK, Müller-Eberhard HJ. Complement C3 convertase: cell surface restriction of β1H control and generation of restriction on neuraminidase-treated cells. Proc Natl Acad Sci U S A 1978;75:2416–2420.
11. Reutter W, Köttgen E, Bauer C, Gerok W. Biological significance of sialic acid. In: Schauer R, editor. Sialic acids: chemistry, metabolism and function (Cell Biology Monograph, vol. 10). New York: Springer-Verlag; 1980. p 263–305.
12. Schauer R, Shukla AK, Schröder C, Müller E. The anti-recognition function of sialic acid: studies with erythrocytes and macrophages. Pure Appl Chem 1984;56:907–921.
13. Tack BF, Prahl JW. Fifth component of human complement: purification from plasma and polypeptide chain structure. Biochemistry 1976;15:4513–4521.
14. Mancini G, Carbonara A, Hermans J. Immunological quantitation of antigens by single radial immunodiffusion. Immunochemistry 1965;2:235–253.
15. Tamerius JD, Pangburn MK, Müller-Eberhard. Detection of a neoantigen on human *i*C3b and C3d by monoclonal antibody. J Immunol 1985;135:2015–2019.
16. Platts-Mills TAE, Ishizaka K. Activation of the alternative path-

way of human complement by rabbit cells. J Immunol 1974;
113:348–358.

17. Harrison RA, Lachmann PJ. Complement technology. In: Weir DM,
editor. Handbook of experimental immunology, 4th edition. Oxford:
Blackwell; 1986. p 5A.1–23.9.

18. Hammond KS, Papermaster DS. Fluorometric assay of sialic acid in
the picomole range: a modification of the thiobarbituric acid assay.
Analyt Biochem 1976;74:292–297.

19. Sugahara K, Sugimoto K, Nomura O, Usui T. Enzymatic assay of se-
rum sialic acid. Clin Chim Acta 1980;108:493–498.

20. Hess EL, Coburn AF, Bates RC, Murphy P. A new method for measur-
ing sialic acid levels in serum and its application to rheumatic fever. J
Clin Invest 1957;36:449–455.

21. Pilatte Y, Bignon J, Lambré CR. Sialic acids as important molecules
in the regulation of the immune system: pathophysiological implica-
tions of sialidases in immunity. Glycobiology 1993;3:201–217.