American Journal of Pathology, Vol. 138, No. 1, January 1991

Urinary Excretion of the C5b-9 Membrane Attack Complex of Complement Is a Marker of Immune Disease Activity in Autologous Immune Complex Nephritis

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The urinary excretion of the C5b-9 membrane attack complex of complement correlates with glomerular deposition of antibody in the passive Heymann nephritis (PHN) model of membranous nephropathy (MN). To determine if this parameter can be correlated with antibody deposition in a model of MN induced by an autologous mechanism and thus more analogous to human MN, the relationship of urinary C5b-9 to ongoing glomerular immune complex formation late in autologous immune complex nephritis (AICN) was studied. Based on urinary C5b-9, the animals were divided into two groups at 12 weeks after induction of AICN, those with persistently high urinary C5b-9 excretion and those in whom urinary excretion of C5b-9 returned to undetectable levels. While all rats developed glomerular deposition of rat IgG and significant proteinuria, high C5b-9 excretors had greater proteinuria and prolonged positive staining for glomerular C3. When normal syngeneic kidneys were transplanted into rats (n = 3) from each group, only those with persistent C5b-9 excretion developed subepithelial immune deposits of rat IgG in the transplanted kidney. As in the PHN model of MN, proteinuria was dissociated widely from urinary C5b-9 excretion, glomerular C3 staining, and evidence of circulating antibody. Thus these findings demonstrate that urinary excretion of C5b-9 serves as an index of on-going immunologic disease activity in the AICN model of MN, while proteinuria does not. (Am J Pathol 1991, 138:203–211)

Membranous nephropathy (MN) is the most common cause of idiopathic nephrotic syndrome in adults.[1] The disease follows a variable and unpredictable course. Some patients undergo spontaneous complete remission, while others with apparently similar levels of proteinuria and glomerular morphology progress to renal failure.[2] Because the disease is mediated by subepithelial deposition of antibody of unknown specificity, therapy generally has used nonspecific suppression of the immune system with steroids and cytotoxic agents.[3,4] However the widely variable and slowly progressive nature of the disease has made assessment of therapeutic benefit difficult.[5] This is complicated further by the observation that proteinuria, the most widely monitored parameter of disease activity, appears to persist in MN long after glomerular antibody deposition ceases.[6–8]

In previous studies using the passive Heymann nephritis (PHN) model of MN in rats mediated by injected heterologous antibody to glomerular epithelial cell membrane antigens, we and others showed that proteinuria is mediated by C5b-9 and involves glomerular epithelial cell membrane insertion of C5b-9 with subsequent endocytosis, transcellular passage, and excretion into the urinary space.[9–13] In the PHN model, urinary C5b-9 excretion is related directly to the amount of glomerular antibody deposition in both the heterologous and autologous phases of the disease and serves as a more sensitive index of disease activity than urinary protein excretion.[14]

In the present study we tried to relate the amount of urinary C5b-9 excretion to disease activity in the autologous immune complex nephritis (AICN) model of MN. Autologous immune complex nephritis is more analogous to human MN than PHN because it is mediated by autologous antibody, simulating the autoimmune process thought to underlie human MN.[15,16] Like human MN, the clinical course

Presented in part at the 21st annual meeting of the American Society of Nephrology, Washington, D.C., December 1988 and subsequently published in abstract form (Kidney Int 1989, 35:359).

Supported in part by United States Public Health Service grants DK 34198, DK 07467, and DK 39068 and by research grants from the Northwest Kidney Foundation and the Lupus Foundation of America.

Accepted for publication August 28, 1990.

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is variable[17] and immunoglobulin deposition progresses slowly over time.[18] The glomerular morphologic changes closely resemble those of chronic human MN.[19] By comparing groups of rats with chronic AICN and nephrotic range proteinuria with and without elevated urinary C5b-9 excretion, we provided additional data in a model of MN to establish the reliability of measurements of urinary C5b-9 excretion as a marker of ongoing glomerular deposition of antibody to glomerular epithelial cell and thus of immunologic disease activity.

Materials and Methods

Experimental Design

Lewis rats with AICN induced by a single footpad injection of Fx1a were subjected to weekly measurements of urinary protein excretion and urinary C5b-9 excretion for 30 weeks. Renal biopsy material was obtained at 20 and 30 weeks. To test the hypothesis that elevated urinary C5b-9 excretion represented on-going glomerular immune deposit formation, rats were divided into two groups based solely on presence or absence of persistently elevated C5b-9 excretion after week 12. This time was chosen because while early in the course of AICN most rats (10 of 12) excreted urinary C5b-9, by week 12 a clear seperation was observed between those rats that continued to excrete large amounts of C5b-9 and those rats in whom urinary C5b-9 had ceased completely. This separation persisted throughout the final 18 weeks of observation. Three rats with persistently elevated urinary C5b-9 excretion and three rats in whom urinary C5b-9 excretion was absent (normal urinary C5b-9) were selected at random to receive a normal syngeneic renal transplant at 30 weeks. The transplanted kidney served as a detector of on-going immune disease activity. These detector kidneys then were biopsied weekly for 4 weeks and studied by quantitative immunofluorescence (IF) densitometry for rat IgG deposits and by electron microscopy for evidence of subepithelial immune deposit formation.

Induction of AICN

Autologous complex nephritis was induced in 200-g Lewis rats (Charles River, Wilmington, MA) by a single injection of Fx1a in complete Freunds adjuvant into each rear foot. The Fx1a antigen was prepared from cortical tissue of Sprague-Dawley rats, as has been described elsewhere.[21]

Measurement of Urinary C5b-9 Excretion

All urine specimens were collected in 10% (vol/vol) buffer containing a mixture of protease inhibitors, including 10 mmol/l (millimolar) benzamidine (Sigma Chemical Co., St. Louis, MO), 10 mmol/l epsilon aminocaproic acid (Sigma), 20 mmol/l ethylenedinitrilo tetraacetic acid (EDTA), and 100 kallikrein inhibitor units/ml of apotinin (Sigma). Detection of C5b-9 was performed by an enzyme-linked immunoabsorbent assay (ELISA) that used two mouse monoclonal antibodies to a neoantigen of C5b-9 (2A1), and to C6 (3G11), which were raised, purified, and characterized as described elsewhere.[12] Briefly, MAbs were identified by their reactivity on immunoblots to rat EDTA plasma and zymosan-activated rat serum separated by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE). Antibodies to human-complement components that demonstrated cross-reactivity with rat proteins were used as references.[12] 3G11 identified the same protein in EDTA plasma as did the antibody to human C6, as well as a second high–molecular-weight band in zymosan-activated serum, presumably representing the C5b-9 complex of complement. 2A1 reacted intensely with this same high–molecular-weight component in zymosan-activated serum but failed to react with EDTA plasma and therefore presumably represents antibody to a C5b-9 neoantigen.[12]

Microtitration plates (NUNC, Roskilda, Denmark) were coated with 4 μg/well (2A1) in carbonate buffer. After blocking with 0.05% casein (Sigma) in phosphate-buffered saline (PBS), serial dilutions of urine samples were incubated in wells overnight at 4°C. After washing with PBS-0.05% Tween 20 (Sigma), biotinylated 3G11, 400 ng/well, was added for 2 hours, followed after washing by horseradish peroxidase coupled to avidin D (Vector, Burlingame, CA). After 1-hour incubation, the wells were washed once again, followed by addition of 100 μl of solutions containing 0.2 mmol/l 2.2′-azine-di-(3-athylbenythiazolinesulfonate) ABTS (Boehringer-Mannheim, Indianapolis, IN) and 3.0 mmol/l H202 in 100 mmol/l acetate buffer at pH 5.0. The extinction at 405 nm was read after 30 minutes at room temperature using Dynatech MR560 microtiterplate reader (Dynatech, Alexandria, VA). C5b-9 units were calculated using previously defined zymosan-activated rat serum reference standard.[12]

Renal Transplantation

All transplants were carried out in syngeneic Lewis rats. The left kidneys of normal rats were harvested using aseptic techniques following induction of anesthesia with equethesin (Drug Services, University of Washington, Seattle, WA). A cuff of vena cava and a segment of aorta were included to facilitate the vascular anastomoses. A generous length of ureter was obtained for direct implantation into the recipient bladder. The kidney was flushed *in situ* with 4°C normal saline containing 0.1 mg/ml lidocaine and 10 units/ml heparin. Topical papaverine was

applied to the renal artery to prevent spasm. The kidney was stored on ice until the recipient was prepared. Preparation consisted of dissection of a length of vena cava and aorta. End-to-side vascular anastomoses were performed using 10-0 monofilament nylon using the microsurgical technique of Silber[21] followed by ureterercystostomy.

Immunofluorescence Studies

All renal biopsies of native kidneys were performed with the animals anesthetized with ether *via* a lateral flank incision. Biopsies obtained from transplanted kidneys were accessed *via* a midline abdominal incision. Hemostasis was achieved with gelfoam (Upjohn, Kalamazoo, MI). The tissue was flash frozen in dry ice-isopentane and stored at −70°C until processed. Cryostat sections were cut, stained, and studied with the fluorescein-conjugated IgG fractions of monospecific antisera to rat IgG, and rat C3 (Cappel Laboratories, Inc., Cochraneville, PA), as described elsewhere.[22] Indirect IF for rat C5b-9 was performed by incubating tissue with an appropriate dilution of biotinylated 2A1 for 1 hour followed by staining with fluorescein-conjugated strepavidin (Amersham, Arlington Heights, IL). Semiquantitative immunofluorescence (IF) evaluations of native AICN kidneys were graded 0, trace, or 1+ to 4+, as described previously.[14]

Quantitative IF densitometry of biopsy material from transplant kidneys was evaluated using a Leitz 560 immunofluorescent microscope (Leitz, Wetzlar, FRG) with visual output connected to a photomultiplier and quantitated. All densitometry readings were done under immersion oil and the biopsy material was magnified 640 times. The densitometry field was fixed such that an average glomerular cross-section extended beyond all borders of the field by 10% to 20%. Whole glomeruli that completely filled the defined field were identified by light microscopy scanning. The photomultiplier was adjusted to assign a value of 80 to the rat C3 IF of a day 5 PHN glomeruli (which we read semiquantitatively as 2+) and a value of 10 to tubular background. Five representative glomeruli from each tissue section were read and averaged. All tissues were cut, stained, and analyzed at a single sitting to minimize changes due to processing of tissue or to intensity of the immunofluorescence source.

Electron Microscopy (EM) Studies

Tissue from transplanted kidneys was placed immediately into half-strength Karnovsky solution and cut into 0.5-mm pieces with two new razor blades. Tissue was preserved in this solution at 4°C until embedding in 504 epon. Sections of 2 μm were cut and scanned by a renal pathologist (CA) without knowledge of tissue code. This observer was told to look specifically for presence of glomerular subepithelial deposits. Representative photomicrographs were obtained on all samples.

Miscellaneous Measurements

Urine protein excretion was measured by a sulfosalicyclic acid method[23] using a whole serum standard (Lab Trol, Dade Diagnostics, Aquado, Puerto Rico). For statistical analysis, all results of 24-hour urine C5b-9 determinations and urine protein measurements are expressed as mean ± standard error.

Results

Development of AICN

All rats (n = 11) developed AICN within 6 to 10 weeks, as shown by urinary protein excretion of more than 30 mg/day (normal urinary protein excretion, less than 10), the typical appearance of deposits of IgG, C3, and C5b-9 by IF. Proteinuria increased to peak at week 13 (279 ± 39 mg/day) and then declined to a low of 117 ± 10 mg/day at week 17. In rats followed from week 12 to 30, proteinuria fluctuated in a sinusoidal pattern on a bimonthly basis (Figure 1). This pattern was demonstrated by individual rats as well as by the group as a whole.

Urinary C5b-9 Excretion

Urinary C5b-9 excretion became elevated in 9 of 11 rats between weeks 6 and 10, as defined as more than than 0.6 units/day, a level that previously we demonstrated to be the lower detection limit of the assay.[12] Levels of urinary C5b-9 varied in periodic evaluations, with intermittent peaks at weeks 13, 17, 20, 25, and 30, which corresponded with peaks in average protein excretion in the group as a whole and in individual rats.

Comparing Elevated C5b-9 Excretion Group with Negative C5b-9 Group

Rats with persistently elevated urinary C5b-9 excretion levels after week 12 are contrasted with rats that had normal C5b-9 excretion levels in Table 1 and Figures 2a and b. Rats that exhibited persistently elevated urinary C5b-9 excretion after week 12 had an earlier onset of proteinuria (week 5 *versus* week 6.5) and a slightly higher time-averaged protein excretion calculated from week of onset to week 30 (239 + 13 mg/day *versus* 154 + 23

mg/day, $P < 0.05$). Both groups exhibited similar oscillations in week-to-week level of urinary protein excretion (Figure 2a). Proteinuria at 20 and 30 weeks was somewhat higher in the urinary C5b-9 excretion group compared to the negative group (Table 1).

Immunofluorescence studies showed identical 3+ granular deposits of rat IgG in both groups at 20 and 30 weeks and identical 1+ glomerular C5b-9 staining. However animals with high urinary C5b-9 excretion had 1–2+ IF staining for C3 at 20 weeks, which persisted at 30 weeks (Table 1), whereas C5b-9–negative rats had trace to negative C3 at 20 weeks and no detectable C3 at 30 weeks (Table 1).

Table 1. *Comparison of Persistently High Urinary C5b-9 Excretors (C5b-9+) with Negative C5b-9 Excretors (C5b-9−)*

	C5b-9+ (n = 6)	C5b-9− (n = 5)
Average onset of proteinuria (wks)	5	6.5
Average urinary protein excretion	239 ± 13	154 ± 23*
Protein excretion		
Week 10	381 ± 54	110 ± 38*
Week 20	208 ± 85	150 ± 25†
Week 30	338 ± 63	258 ± 56†
IF of glomeruli for RIgG		
Week 20	3+	3+
Week 30	3+	3+
IF of glomeruli for C3		
Week 20	1–2+	tr-neg
Week 30	1+	neg

* $P < 0.05$; † $P < 0.10$.
IF, immunofluorescence.

Renal Transplant Study

To determine if persistently elevated urinary C5b-9 excretion reflected active disease, three rats in each group underwent syngeneic renal transplantation at week 30. The characteristics of the recipients are presented in Table 2. Recipients reflected the general differences observed between persistent C5b-9 excretors and nonexcretors. All had 3–4+ glomerular deposition of rat IgG, 1+ glomerular C5b-9, and significant proteinuria, although C5b-9 excretors had somewhat more proteinuria. As previously mentioned, only C5b-9 excretors showed IF evidence of C3.

Sequential biopsies of the transplanted kidneys from rats with negative C5b-9 excretion were never positive for rat IgG by IF, nor could subepithelial deposits be identified by EM. In contrast, rats with elevated urinary C5b-9 excretion developed finely granular deposits of rat IgG at 1 week, with a steady increase during the next 3 weeks as assessed by quantitative IF densitometry (Figure 3). Electron microscopy at 3 weeks confirmed the presence of subepithelial deposits in recipients that were elevated urinary C5b-9 excretors (Figure 4). Electron microscopic survey of glomerular tissue obtained at 3 weeks from the transplanted kidneys in C5b-9–negative animals revealed no deposits (Figure 4).

Discussion

The findings reported here provide strong evidence that increased urinary C5b-9 excretion is an accurate measure of disease activity in markedly proteinuric rats in whom the AICN model of MN has been established, when disease activity is defined as continuing formation of immune deposits in glomeruli. Conclusive proof of this hypothesis would require correlation of urinary C5b-9 excretion with deposition of a specific nephritogenic antibody. However, in AICN, the exact nephritogenic antigen–antibody system has not been well defined.

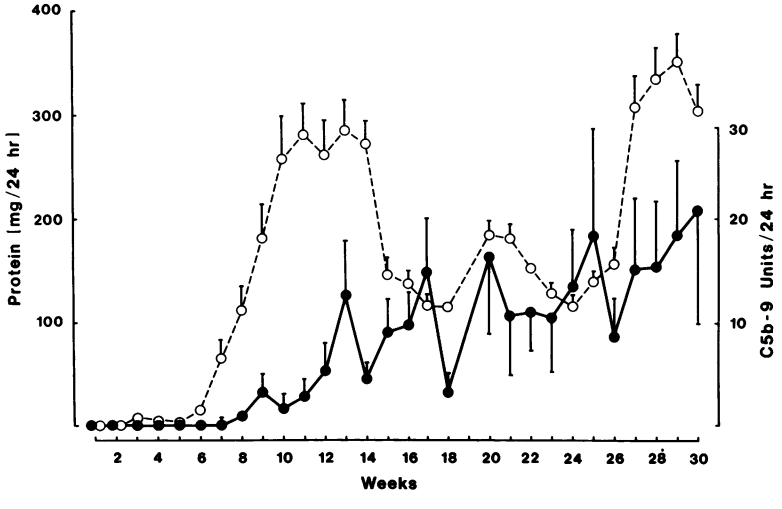

Figure 1. *Average weekly urinary C5b-9 (closed circles) and protein excretion (open circles) of all rats. The periodic nature of both urinary protein and urinary C5b-9 was noted in the group of rats as a whole and in each individual rat.*

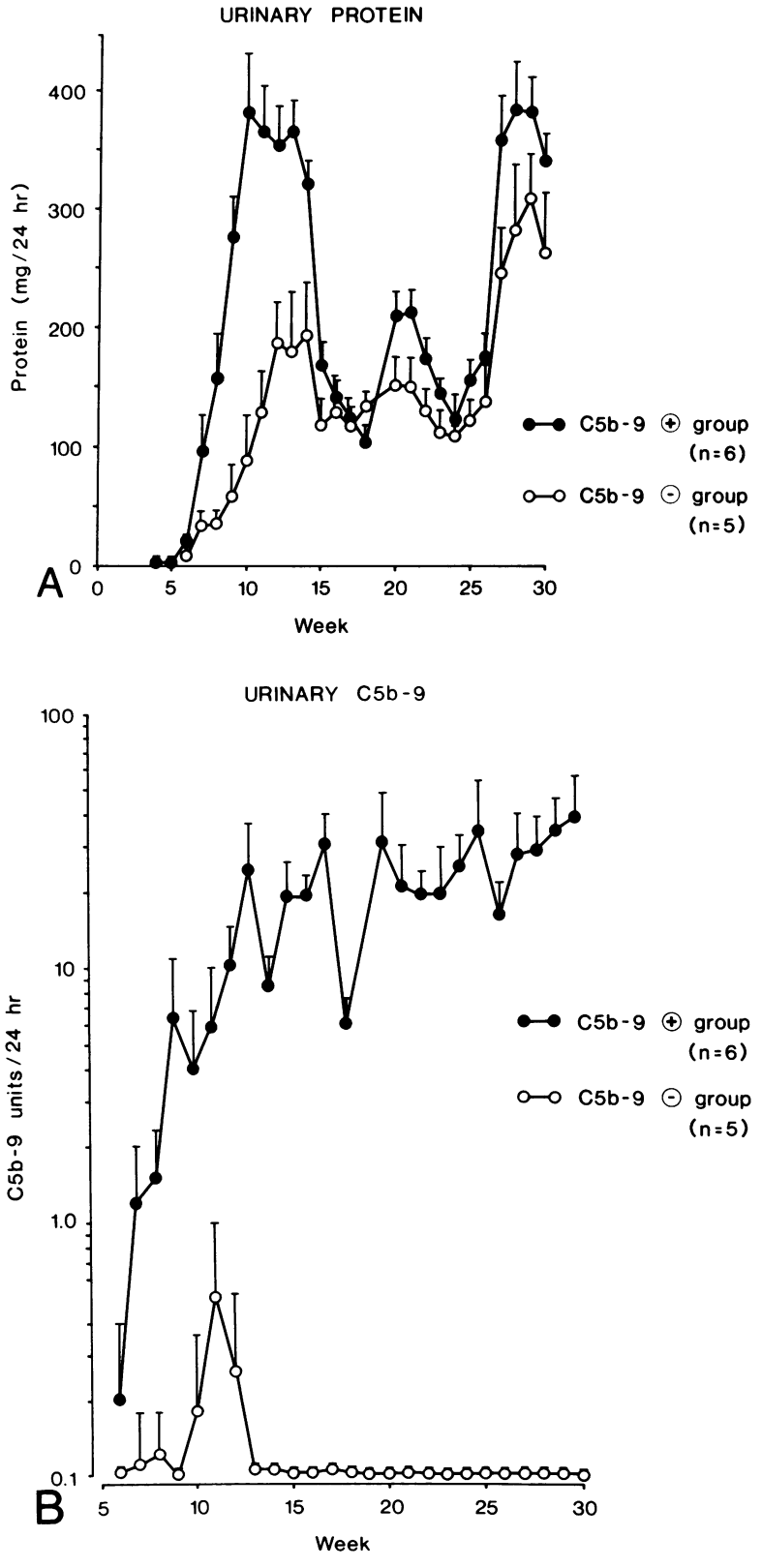

Figure 2. *Comparison of weekly urinary protein excretion* (a) *and urinary C5b-9 excretion* (b) *between the two groups. Persistant urinary C5b-9 excretors (n = 6) (closed circles) are contrasted with AICN rats in whom urinary C5b-9 has returned to zero (n = 5) (open circles).*

Evidence has been presented to implicate glomerular epithelial cell membrane antigens, particularly gp330, various circulating tubular antigens, and anti-idiotypic antibodies that may form glomerular deposits through internal imaging or result in circulating immune complex formation and deposition.[24-26]

The situation in human membranous nephropathy appears similar, although most recent evidence suggests

Table 2. *Comparison of Individual Rats with High and Low Urinary C5b-9 Levels Before Transplantation*

	C5b-9+			C5b-9−		
Rat	1	2	3	4	5	6
Average proteinuria (mg)	290	220	260	173	192	201
Onset of proteinuria (wks)	6	7	8	9	8	9
Proteinuria						
Week 10	596	276	399	115	215	50
Week 20	286	169	235	158	194	169
Week 30	426	341	398	283	356	295
Glomerular RIgG						
Week 20	3+	3+	3+	3+	3+	3+
Week 30	3+	3+	3+	3+	3+	3+
Glomerular C3						
Week 20	2+	1+	1+	tr	tr	—
Week 30	1+	1+	tr	—	—	—

an autoimmune mechanism involving antibodies to glomerular epithelial cell membrane antigens.[16,27,28] It is probable that in both the rat and human diseases that more than one antigen–antibody system is involved. In the absence of a specific nephritogenic antibody response to monitor, assessment of disease activity is necessarily indirect. In this study, normal detector kidneys transplanted into proteinuric recipients with AICN were used to document on-going disease activity. Our results clearly show that rats with elevated urinary C5b-9 excretion are continuing to form glomerular immune deposits, whereas rats with similar glomerular lesions and levels of protein excretion but without detectable urinary C5b-9 appear to have no active disease as judged by the failure to form subepithelial deposits in transplanted detector kidneys. A similar heterogeneity in chronic AICN rats was reported by Edgington et al,[18] who found that 4 of 17 rats with AICN at 15 weeks failed to develop deposits in normal transplanted kidneys.

In previous studies using the PHN model of MN induced with an injected heterologous antibody to Fx1A, we showed that urinary C5b-9 excretion is a specific marker of disease induced by antibody to glomerular epithelial cell membrane antigens,[12] does not occur when subepithelial immune deposits are induced by other mechanisms,[12] parallels the quantity of deposits formed in both the heterologous and autologous phases of disease,[14] and ceases immediately when on-going immune deposit formation is halted by transplanting nephritic kidneys into normal recipients, despite persistence of proteinuria.[14] The present study extends these observations in several ways. Documentation that urinary C5b-9 excretion also is elevated in AICN provides evidence that the mechanism involving antibody to glomerular epithelial cell antigens resulting in subepithelial immune deposits is apparently a major mechanism of immune deposit formation in this model as well, an hypothesis that has not been accepted uniformly (reviewed in Couser and Abrass[16]). Furthermore the AICN model is probably more analogous to human idiopathic membranous nephropathy than is PHN because of the insidious rather than abrupt onset of disease, variability among animals in severity and duration of disease, and protracted course. Finally the results clearly show a phase of chronic persistent proteinuria in the absence of on-going disease activity that we believe is probably analogous to the situation observed in humans.

Our finding that proteinuria persists in a subset of animals without evidence of on-going disease confirms our earlier studies in PHN[14] and studies by others in AICN[29,30] documenting that the alterations in glomerular barrier function that occur in MN are not readily reversible and persist long after disease activity ceases. In parallel studies conducted in patients with membranous nephropathy and other diseases, we showed that C5b-9 excretion is elevated in a subset of patients with idiopathic membranous nephropathy and nephrotic range proteinuria.[31] Furthermore patients with elevated urinary C5b-9 levels are detected earlier in the course of disease and exhibit higher levels of urine protein excretion, characteristics that are consistent with a higher likelihood of active disease.[31] Thus the ability to detect on-going disease activity in membranous nephropathy indirectly by measuring urinary C5b-9 excretion may have significant implications as a guide to administration of potentially toxic immunosuppressive therapy to patients with idiopathic nephrotic syndrome and membranous nephropathy.

These findings confirm the relative insensitivity of urine protein excretion as an index of disease activity. They also support our earlier findings in PHN that glomerular staining for C3 closely parallels urinary C5b-9 excretion and therefore also may serve as a marker of disease activity in biopsy specimens.[14] As previously reported in PHN, we found that all AICN rats had evidence of glomerular C3 activation in the early stages of disease. However complete C3 disappearance was noted in the group judged to be inactive by absence of urinary C5b-9 and failure to form deposits after transplantation, while C3 persisted in those rats that were shown to have active disease. Observations by us[32] and others[33] demonstrated the rapid clearance of glomerular C3 deposits when complement

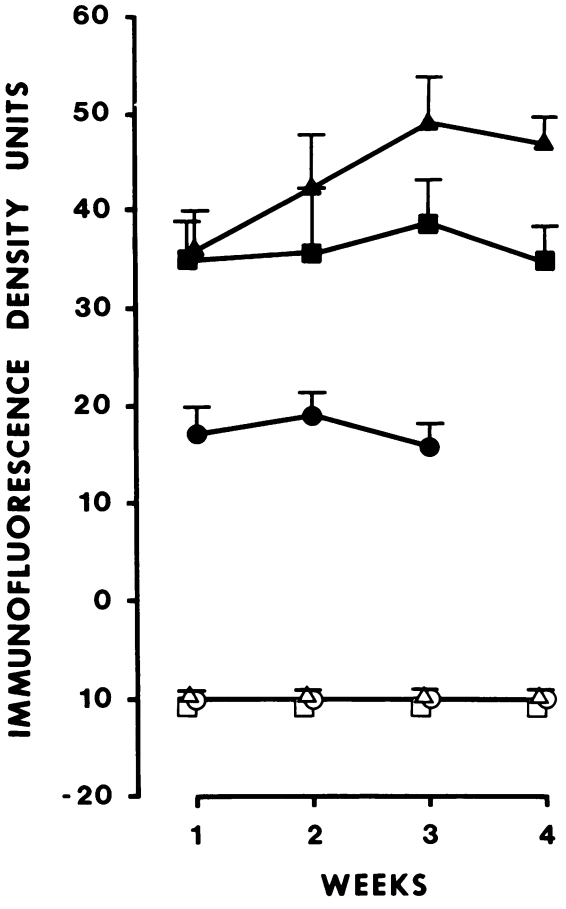

Figure 3. *Appearance of rat IgG in transplanted normal kidneys. Quantitative immunofluorescence densitometry readings displayed as a function of time after transplantation. Closed triangle, square, and circle correspond to C5b-9+ rats 1, 2, and 3; open symbols correspond to C5b-9- rats 4, 5, and 6. All three C5b-9+ rats developed deposits as demonstrated by increasing RIgG immunofluorescence density, while three C5b-9- rats remain negative. Derivation of density units is described in the text.*

activation is terminated by systemic complement depletion. Thus the presence of C3 staining by IF strongly suggests on-going deposit formation, and C3 disappearance late in the course of AICN in certain animals suggests that deposit formation ceased. These findings also correlate with the earlier work of Noble, who suggested that complement fixing antibody deposition was necessary for the development of proteinuria in AICN.[17,34]

We are intrigued by the data presented in Figure 1 that document a waxing and waning of urinary excretion of both protein and C5b-9 after 12 weeks in AICN. This sinusoidal pattern of oscillation about an 8-week mean was observed in individual animals as well as in the group as a whole and has not been discussed previously. The implication of this finding is that severity of the underlying immunologic disease mechanism may oscillate in a similar manner. The mechanism for such an oscillation is not immediately apparent and no further studies were done to investigate this. It has been suggested that anti-idiotypic antibody production may occur late in AICN and contribute to formation of glomerular immune deposits.[25] It is conceivable that this variation in disease activity could represent alterations in B-cell activity and antibody production under anti-idiotypic antibody control.[25]

The relationship between the increased urinary C5b-9 excretion and the mechanism of proteinuria in AICN remains undefined. In previous studies we showed that C5b-9 does not appear to derive from filtered circulating C5b-9 complexes or from antibody reacting with tubular brush-border antigens in proteinuric animals.[12] In humans, some urinary C5b-9 formation appears to occur spontaneously in the bladder when high protein concentrations are present and is limited by the amount of C5,[31] but previous studies in several proteinuric rat models indicate that spontaneous C5b-9 generation does not appear to occur in the rat.[12] We believe that urinary C5b-9 represents membrane-inserted C5b-9, which is derived as a consequence of complement activation from the interaction of antibody with antigens expressed on the glomerular epithelial cell membrane.[12] After membrane insertion, C5b-9 complexes are engulfed by the cell, transported intracellularly, and exocytosed into the urinary space.[13] Although urinary C5b-9 may contain S-protein, a circulating protein that binds to active C5b-9 and prevents cell membrane insertion,[35,36] it is likely that S-protein binding may occur after exocytosis of membrane-inserted C5b-9 complexes into the urine.[37] Although C5b-9 was shown clearly to be the principal mediator of proteinuria in experimental membranous nephropathy (reviewed in Scott et al[9]), the cellular mechanism is unclear. Studies by others have shown that sublytic C5b-9 attack on the epithelial cell can result in cell activation as assessed by an increase in cytosolic Ca2+, activation of phospholipase C, and production of inositol triphosphate and 1,2 diacylglycerol.[38] The consequences of cell activation by sublytic C5b-9 include an increase in epithelial cell production of prostaglandins and type IV collagen.[39,40] However the exact relationship between these phenomena and the loss of glomerular barrier function remains to be defined.

Our findings document an increase in urinary C5b-9 excretion in the AICN model of membranous nephropathy, thereby implicating an antibody to glomerular epithelial cell membrane antigens in the pathogenesis of subepithelial immune deposit formation and a role for C5b-9 in the development of proteinuria in this classical animal model of human membranous nephropathy. Our studies show further that urinary C5b-9 excretion (and glomerular C3 deposition by immunofluorescence) accurately reflect on-going disease activity as documented by the development of immune deposits in transplanted detector kidneys. The persistence of nephrotic-range proteinuria and glomerular morphologic changes in the absence of disease activity as assessed here suggest that urinary C5b-

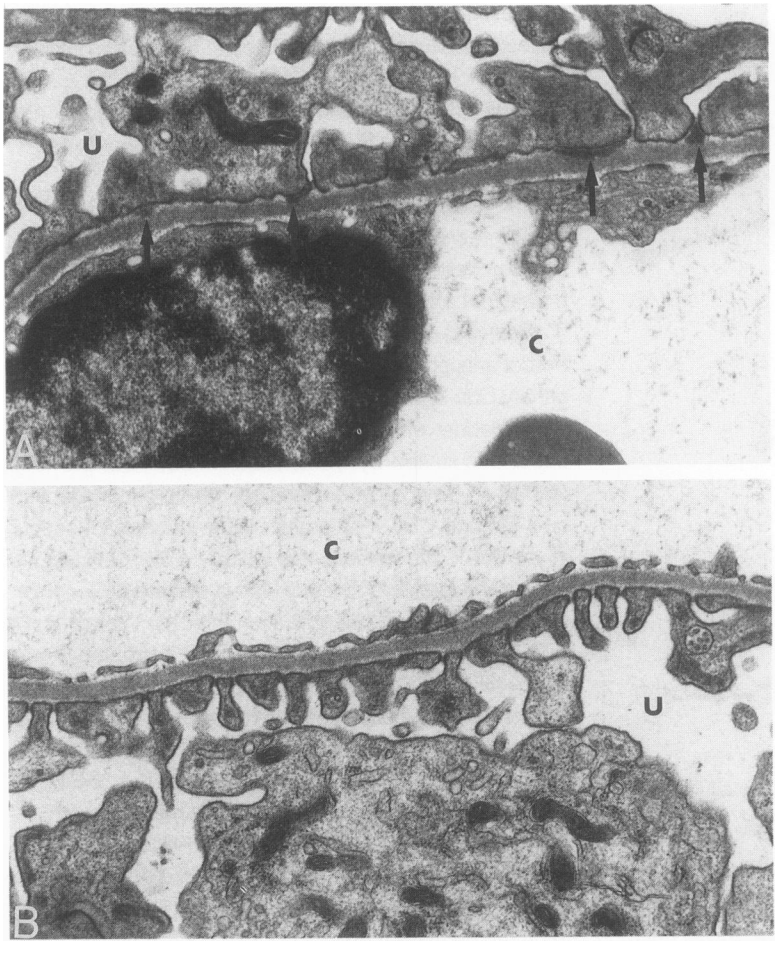

Figure 4. *Electron microscopy of representative transplant kidneys into a C5b-9+ rat* **(A)** *and into a C5b-9− rat* **(B)**. *Subepithelial immune deposits demonstrated with arrows are present exclusively in the glomeruli of C5b-9+ rats. Magnification of both micrographs is 23,000×. U, urinary space; C, capillary lumen.*

9 excretion may be useful as an index of immunologic disease activity in this experimental model.

Acknowledgments

The authors thank Lloyd Aberle for technical assistance and Maggie Whitcomb for preparing the manuscript.

References

1. Couser WG: Glomerular disorders, Chapter 80. *In* Wyngaarden JB, eds. Cecil Textbook of Medicine, 17th Edition. Philadelphia, WB Saunders, 1985, pp 568–588
2. Davidson AM, Cameron JS, Kerr DNS, Ogg CS, Wilkinson RW: The natural history of renal function in untreated idiopathic membranous glomerulonephritis in adults. Clin Nephrol 1984, 22:61–67
3. Collaborative Study of the Adult Idiopathic Nephrotic Syndrome: A controled study of short-term prednisone treatment in adults with membranous nephropathy. N Engl J Med 1979, 301:1301–1306
4. Cattran DC, Delmore T, Roscoe J, Cole E, Cardella C, Charron R, Ritchie S: The Toronto glomerulonephritis study group: A randomized trial of prednisone in patients with idiopathic membranous nephropathy. N Engl J Med 1989, 320:210–215
5. Ponticelli C, Zucchelli P, Cagnoli L, Cesana B, Pozzi C, Pasquali S, Imbasciati E, Grassi C, Redaelli B, Sasdelli M, Locatelli F: A randomized trial of methylprednisolone and chloramphenicol in idiopathic membranous nephropathy. N Engl J Med 1989, 320:8–13
6. Donadio JV, Torres VE, Velosa JA, Wagoner RD, Holley KE, Okamura M, Ilstrup DM, Chu C: Idiopathic membranous nephropathy: The natural history of untreated patients. Kidney Int 1988, 33:708–715
7. Tornroth T, Skrifars B: The development and resolution of glomerular basement membrane changes associated with subepithelial immune deposits. Am J Pathol 1975, 79:219–230
8. Hoorntje SJ, Weening JJ, The TH, Kallenberg CGM, Donker ABJM, Hoedemaeker PJ: Immune-complex glomerulopathy in patients treated with captopril. Lancet 1980, i:1212–1215
9. Couser WG, Baker PJ, Adler S: Complement and the direct mediation of immune glomerular injury: A new perspective. Kidney Int 1985, 28:879–890
10. Baker PJ, Ochi RF, Schulze M, Johnson RJ, Campbell C,

Couser WG: Depletion of C6 prevents development of proteinuria in experimental membranous nephropathy in rats. Am J Pathol 1989, 135:185–194

11. Cybulsky AV, Rennke HG, Reintzeig ID, Salant DJ: Complement-induced glomerular epithelial cell injury. Role of the membrane attack complex in rat membranous nephropathy. J Clin Invest 1986, 77:1096–1107
12. Schulze M, Baker PJ, Perkinson DJ, Johnson RJ, Ochi RF, Stahl RAK, Couser WG: Increased urinary excretion of C5b-9 distinguishes passive Heymann nephritis from other forms of experimental glomerulonephritis in the rat. Kidney Int 1989, 35:60–68
13. Kerjaschki D, Schulze M, Binder S, Kain R, Ojha PP, Susani M, Horvat R, Baker PJ, Couser WG: Trancellular transport and membrane insertion of the C5b-9 membrane attack complex of complement by glomerular epithelial cells in experimental membranous nephropathy. J Immunol 1989, 143: 546–552
14. Pruchno CJ, Burns MW, Schulze M, Johnson RJ, Baker PJ, Couser WG: Urinary excretion of C5b-9 reflects disease activity in passive Heymann nephritis. Kidney Int 1989, 36:65–71
15. Edginton TS, Glassock RJ, Dixon FJ: Autologous immune complex pathogenesis of experimental allergic glomerulonephritis. Science 1967, 155:1432–1434
16. Couser WG, Abrass CK: Pathogenesis of membranous nephropathy. Ann Rev Med 1988, 39:517–530
17. Noble B, Van Liew JB, Andres GA, Brentjens JR: Factors influencing susceptibility of LEW rats to Heymann nephritis. Clin Immunol Immunopathol 1984, 30:241–254
18. Edginton TS, Lee S, Dixon FJ: Persistence of the autoimmune pathogenetic process in experimental autologous immune complex nephritis. J Immunol 1969, 103:528–536
19. Heymann W, Hackel DB, Harwood J, Wilson SGG, Hunter JLP: Production of the nephrotoxic syndrome in rats by Freund's adjuvant and rat kidney suspensions. Proc Soc Exp Biol Med 1959, 100:660–664
20. Salant DJ, Darby C, Couser WG: Experimental membranous glomerulonephritis in rats. Quantitative studies of glomerular immune deposit formation in isolated glomeruli and whole animals. J Clin Invest 1980, 66:71–81
21. Silber SJ, Crudop: Kidney transplantation in inbred rats. Am J Surg 1973, 125:551–553
22. Couser WG, Stilmant MM, Darby C: Autologous immune complex nephropathy. I. Sequential study of immune complex deposition, ultrastructural changes, proteinuria and alterations in glomerular sialoprotein. Lab Invest 1976, 34:23–30
23. Bradley GM, Benson ES: Examination of the urine. *In* Davidson I, Henry JB, eds. Todd Sanford Clinical Diagnosis by Laboratory Methods, 15th Edition. Philadelphia, WB Saunders, 1974, p 74
24. Singh AK, Makker SP: Circulatory antigens of Heymann nephritis. I. Identification and partial characterization. Immunology 1986, 57:467–472
25. Abrass CK: Evaluation of sequential glomerular eluates from rats with Heymann nephritis. J Immunol 1986, 137:530–535
26. Kerjaschki D, Farquhar MG: The pathogenic antigen of Heymann nephritis is a membrane glycoprotein of the renal proximal tubule brush border. Proc Nat Acad Sci (USA) 1982, 79:5557–5561
27. Niles J, Collins B, Baird L, Erikson M, Bradford D, Pan G, Hsiung CK, Schneeberger E, Bhan A, McCluskey R: Antibodies reactive with a renal glycoprotein and with deposits in membranous nephritis. Kidney Int 1987, 31:338(Abstr)
28. Makker SP, Kanalas JJ: Autoantibodies to human GP330 in sera of patients with idiopathic membranous glomerulonephropathy. Kidney Int 1989, 35:211(Abstr)
29. Lewis EJ, Bolton WK, Spargo BA, Stuart FP: Persistent proteinuria in the rat with Heymann nephritis. Clin Res 1972, 20:763(Abstr)
30. Makker SP, Kanalas JJ: Course of transplanted Heymann nephritis kidney in normal host. Implications for mechanism of proteinuria in membranous glomerulonephropathy. J Immunol 1989, 142:3406–3410
31. 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 complement C5b-9 complex in a subset of patients with membranous nephropathy. N Engl J Med (Submitted for publication)
32. Schulze M, Burns M, Pruchno C, Baker P, Johnson R, Couser W: Glomerular C3c deposition reflects active immune disease in experimental glomerulonephritis. Kidney Int 1989, 35: 362(Abstr)
33. Noble B, Andres GA, Brentjens JR: Passively transferred anti-brush border antibodies induce injury of proximal tubules in the absence of complement. Clin Exp Immunol 1984, 56: 281–288
34. Noble B, Van Liew JB, Brentjens JR, Andres GA: Effect of reimmunization with Fx1a late in the course of Heymann nephritis. Lab Invest 1982, 47:427–436
35. Mollnes TE, Lachmann PJ: Regulation of complement. Scand J Immunol 1988, 27:127–142
36. Cosyns JP, Kazatchkine MD, Bhakdi S, Mandet C, Grossetete J, Hinglais, Bariety J: Immunohistochemical analysis of C3 cleavage fragments, factor H, and the C5b-9 terminal complex of complement in de novo membranous glomerulonephritis occuring in patients with renal transplant. Clin Nephrol 1986, 26:203–208
37. Bhakdi S, Kaflein R, Halstensen TS, Hugo F, Preissner KT, Mollnes TE: Complement S-protein (vitronectin) is associated with cytolytic membrane-bound C5b-9 complexes. Clin Exp Immunol 1988, 74:459–464
38. Cybulsky AV, Salant DJ, Quigg RJ, Badalamenti J, Bonventre JV: Complement C5b-9 complex activates phospholipases in glomerular epithelial cells. Am J Physiol 1989, 257:F826–F836
39. Hansch GM, Torbohm I, Rother K: Chronic glomerulonephritis: Inflammatory mediators stimulate the collagen synthesis in glomerular epithelial cells. Int Archs Allergy Appl Immunol 1989, 88:139–143
40. Hansch GM, Betz M, Gunther J, Rother KO and Sterzel B: The complement membrane attack complex stimulates the prostanoid production of cultured glomerular epithelial cells. Int Archs Allergy Appl Immunol 1988, 85:87–93