Kidney International, Vol. 50 (1996), pp. 2054–2062

Beneficial effects of systemic immunoglobulin in experimental membranous nephropathy

MASAOMI NANGAKU, JEFF PIPPIN, CATHERINE A. RICHARDSON, MATTHIAS SCHULZE, BESSIE A. YOUNG, CHARLES E. ALPERS, KATHERINE L. GORDON, RICHARD J. JOHNSON, and WILLIAM G. COUSER

Division of Nephrology, Department of Medicine, and Department of Pathology, University of Washington, Seattle, Washington, USA, and Medizinische Hochschule Hannover, Abteilung Nephrologie, Hannover, Germany

Beneficial effects of systemic immunoglobulin in experimental membranous nephropathy. To test the hypothesis that systemic administration of immunoglobulin might reduce glomerular injury in membranous nephropathy through mechanisms involving inhibition of complement activation, we studied the passive Heymann nephritis (PHN) model of membranous nephropathy in rats. The daily administration of immunoglobulin goat IgG (600 mg/kg i.p.) reduced proteinuria by 52%. Quantitative immunohistochemical analysis showed that the glomerular deposition of C3c, an indicator of ongoing complement attack, and of C5b-9 was significantly decreased in the immunoglobulin treated group, while deposition of anti-Fx1A was not affected. Electron microscopic analysis demonstrated that the extent of subepithelial immune complexes did not appreciably differ between treated and control animals. Systemic complement levels were not altered by immunoglobulin treatment. These data suggest that the reduction in proteinuria that resulted from systemic immunoglobulin administration was mediated by modifying the effect of complement induced glomerular injury. This interpretation was further supported by *in vitro* data that documented a significant reduction in C5b-9 induced glomerular epithelial cell lysis in the presence of both goat and rat IgG. These results indicate that systemic administration of immunoglobulin can substantially reduce ongoing complement activation in the glomerulus in PHN rats and that this effect is associated with a significant reduction in glomerular injury.

Membranous nephropathy (MN) is a common cause of idiopathic nephrotic syndrome in adults [1, 2]. Many patients with MN progress to renal failure [3–6]. Treatment of MN remains controversial [7–9]. Several studies have shown no benefit from conventional steroid and immunosuppressive agents [10–13]. Other investigators have reported modest success with regimens employing alternating monthly cycles of intravenous steroids and chlorambucil [14], but toxicity is significant, particularly in patients with impaired renal function [15].

Glomerular injury in MN is believed to be a complement-mediated process because of the remarkable similarity between the functional and immunopathologic features of MN in humans and those of the Heymann nephritis models in rats [16–18]. In passive Heymann nephritis (PHN), subepithelial deposits occur as a consequence of antibody binding to antigens expressed on the surface of the glomerular epithelial cell (GEC), and proteinuria results from GEC membrane insertion of the C5b-9 membrane attack complex of complement [17, 19, 20]. Similar mechanisms may be operative in humans as well [16].

Considerable evidence has now accumulated that intravenous immunoglobulin (IVIG) has beneficial effects on a variety of antibody-mediated diseases in humans including lupus nephritis, IgA nephropathy and ANCA-positive vasculitis [21–23]. In one study, IVIG reduced proteinuria and improved renal function in patients with MN resistant to other therapies [24]. The mechanism by which IVIG exerts a beneficial effect in these diseases is unknown, although most authors have suggested an effect on the immune response probably through idiotype–anti-idiotype interactions [25–27]. However, recent studies demonstrate that IVIG is also a potent inhibitor of complement activation through direct binding to C3 and C4 activation products [28–31]. Based on these observations, we postulated that systemic administration of immunoglobulin (SIG) might be an effective therapy in MN because of its complement inhibitory properties. We tested this hypothesis in the PHN model of MN in rats where the passive administration of nephritogenic antibody precludes any influence of systemic immunoglobulin on the immune response, and no mediators of glomerular injury other than complement have been identified. The results indicate that SIG does substantially reduce evidence of ongoing complement activation in the glomerulus and that this effect is associated with a significant reduction in glomerular injury as measured by urine protein excretion.

Methods

Induction of passive Heymann nephritis and experimental design

The passive Heymann nephritis (PHN) model was induced in 250 g male Sprague-Dawley rats ($N = 12$) (Simonsen Laboratories) by the injection of 0.9 ml of sheep anti-rat Fx1A antiserum as described previously [32, 33]. The injection of nephritogenic antiserum was performed two hours after the initial injection of high dose IgG or vehicle only. An experimental group of 6 rats was treated with IgG, 600 mg/kg i.p., administered two hours before anti-Fx1A antibody injection and 24, 48, and 72 hours later. A control group received the same dose of anti-Fx1A but was treated with vehicle.

Urine protein excretion was measured from days 4 to 5 after the antibody injection using the sulfosalicylic acid method [34], at

Received for publication March 26, 1996
and in revised form July 11, 1996
Accepted for publication July 15, 1996

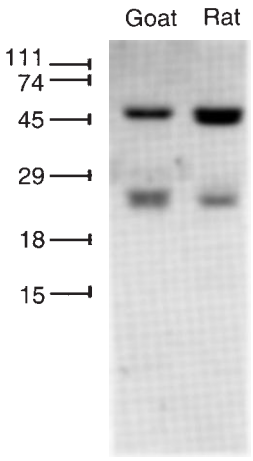

Fig. 1. *CBB staining of purified IgG. Thirty μg of purified IgG was electrophoresed with 15% SDS-PAGE gel and was stained with coomassie brilliant blue, which demonstrated high purity of the protein.*

which time the animals were sacrificed and renal biopsies were obtained for examination by light microscopy, immunofluorescence, and electron microscopy. All animal studies were performed in an accredited animal care facility in accord with the NIH guide lines for the Care and Use of Laboratory Animals.

Preparation of IgG

Goat plasma was purchased from J.J.J. Farms (Redmond, WA, USA). Rat serum was purchased from Sigma (St. Louis, MO, USA). IgG was purified utilizing a caprylic acid precipitation method [35]. After adjusting the pH of the plasma to 5.5 with HCl, 5.5 ml/100 ml plasma of caprylic acid solution (Sigma) was added dropwise with vigorous stirring. Then the solution was stirred for one hour at room temperature and centrifuged at 14K rpm for 30 minutes at room temperature. The supernatant was collected and dialyzed against water which was adjusted with HCl to pH 4.2 in order to enhance its monomer content and stability [36]. The dialyzed solution was centrifuged at 14K rpm for 30 minutes at 4°C. This supernatant was sterilized by filtration and stored at 4°C. If necessary, IgG solution was concentrated utilizing centri-plus (Amicon, Beverly, MA, USA). Purity of the prepared IgG was confirmed by Coomassie brilliant blue staining of 15% SDS-PAGE gel (Fig. 1).

Measurement of goat IgG in serum

Serum levels of goat IgG were measured by radial immunodiffusion (RID) [37] two hours after the injection and 24, 48, 72, 96, and 120 hours later. Briefly, 1.5% agarose in 0.5 M veronal buffer containing a 1:50 dilution of rabbit anti-goat IgG (Cappel, Durham, NC, USA) was poured, and after cooling 2 mm holes were punched. Then 3 μl of goat IgG standards at 5, 2.5, 1.25, 0.625, or 0.313 mg/ml as well as 3 μl of rat plasma at specific time points were added. The diameters of the rings from precipitation of goat IgG in the plasma samples by the antibody were read off of the curve generated by the diameters of the rings from the known standards.

Immunohistochemistry

Renal tissue was embedded in O.C.T., snap-frozen in liquid nitrogen cooled isopentane, and stored at −70°C until processed. Four micron cryostat sections were cut and fixed with 1:1 ethanol/ether. Sections were stained with FITC-conjugated goat anti-rat C3 (Cappel) which reacts primarily with rat C3c [38], biotinylated monoclonal antibody to a neoantigen of rat C5b-9 (2A1), or goat anti-sheep IgG (Cappel).

Quantification of glomerular deposits of antibody and complement by laser scanning confocal microscopy

To examine an effect of SIG on glomerular antibody deposition, ACAS Ultima Interactive Laser Cytometer (Meridian Instruments, Okemos, MI, USA) was utilized for quantitative analysis of immunofluorescence intensity as previously described [39]. Briefly, 4 μm sections of frozen kidney were stained as described above and were then scanned. An image field of 3.2 mm^2 was scanned for each section; where less than 15 glomeruli were present, two fields were scanned. Using the Meridian image analysis system, the fluorescence intensity of individual glomeruli was then measured. Two different sections (containing a total of ~14 to ~52 glomeruli) from each rat were analyzed, and more than 250 glomeruli were measured for each experiment.

Electron microscopy

Tissue for electron microscopy (EM) was fixed in half-strength Karnovsky's solution (1% paraformaldehyde and 1.25% glutaraldehyde in 0.1 M Na cacodylate buffer, pH 7.0), postfixed in osmium tetroxide, dehydrated in graded ethanols, and embedded in epoxy resin. Thin sections were stained with uranyl acetate and lead citrate and examined with a Philips 410 (Philips Export BV, Eindhoven, The Netherlands) electron microscope.

Serum C3, CH50, and BUN measurement

To examine the effect of SIG on serum complement levels, C3 was measured using the radial immunodiffusion method as described above using goat anti-rat C3 (Cappel).

The hemolytic activity of complement in SIG and vehicle treated serum was measured by the hemolysis of sheep erythrocytes sensitized by specific antibodies (anti-sheep hemolysin) and compared to serum taken from the rats prior to therapy [40]. The degree of hemolysis is expressed in CH50 units. One CH50 unit is defined as the volume of serum that will lyse 50% of the erythrocytes in the reaction mixture. The number of CH50 units in 1 ml of serum is the hemolytic titer.

BUN was determined colorimetrically utilizing a commercial kit for the measurement of urea nitrogen (Sigma Diagnostics).

Urinary C5b-9 measurement

Urine was collected overnight (days 4 to 5) into a concentrated mixture of protease inhibitors and rat C5b-9 was assayed using an enzyme-linked immunoabsorbent assay as reported previously [32, 38, 41]. Briefly, a monoclonal antibody to a neoantigen of rat C5b-9 (2A1) was employed as a capturing antibody, while a biotinylated polyclonal antibody to human C6 (Calbiochem, San Diego, CA, USA) was used as a detecting antibody. C5b-9 units were calculated using a previously defined inulin-activated rat serum reference standard.

Studies of GEC in culture

To further examine the effect of IgG on C5b-9 mediated injury to the GEC, a rat glomerular epithelial cell line was established and maintained as previously described [42]. After the 20th passage on collagen matrix (Vitrogen Collagen, Palo Alto, CA, USA), cells were grown on 100-mm tissue culture dishes coated with bovine type I collagen (Collaborative Research, Bedford, MA, USA). They were maintained in K-1 medium supplemented with 2% NuSerum (Collaborative Research) at final concentrations of 5 μg/ml insulin, 5 μg/ml transferrin, and 5 ng/ml selenium (Collaborative Research). The characterization to confirm that this cell line represents glomerular epithelial cells was performed as described previously [43]. Cultured cells exhibited characteristics typical of GEC with positive staining for anti-Fx1A and negative staining for α-smooth muscle actin, Thy 1.1, and factor VIII.

Complement-mediated cell lysis assay

Complement-mediated GEC lysis assays were performed as previously described [44]. Briefly, GEC were passaged into 24-well plates and grown until subconfluent. After washing with veronal buffered saline (VBS), the cells were sensitized with 3 mg/ml of anti-Fx1A for 30 minutes at room temperature. Following further washing, the cells were incubated with 10% normal rat serum in DMEM as a complement source and various concentrations of rat IgG for 90 minutes at 37°C. Similar studies were also done with goat IgG. Cell lysis was quantitated by a lactate dehydrogenase (LDH) assay (Sigma Diagnostics) from supernatants of treated cells. After the assay, all cells were then lysed with 2% Triton X-100, and complement-mediated cell lysis was calculated as the lysis induced by antibody and complement divided by the lysis induced by Triton X-100 multiplied by 100.

Statistics

All results are presented as mean ± SD. Significance was assessed by ANOVA-repeated measures.

Results

Serum level of goat IgG after the treatment

To document that elevated levels of goat IgG in serum were achieved following i.p. injection, we measured serum levels of goat IgG by RID. Two hours after SIG treatment, the serum concentration of goat IgG increased to 4.03 ± 0.39 mg/ml. Serum levels of goat IgG were maintained between 3.5 and 5.0 mg/ml throughout the experimental period.

Proteinuria was attenuated by SIG

Before the induction of PHN, both control and IgG-treated rats showed normal amount of urinary protein excretion (8.0 ± 4.2 and 6.5 ± 2.8 mg/24 hr, respectively). At day 5 of PHN, urinary protein excretion of control rats was 60.6 ± 26.1 mg/24 hr. However, IgG treatment significantly reduced proteinuria to 29.3 ± 24.0 mg/24 hr ($P < 0.05$).

Immunohistochemical analysis

To investigate the mechanism by which SIG reduced proteinuria, we performed immunohistochemical studies. First, we determined if there was a difference in the amount or distribution of anti-Fx1A antibody deposition between control and IgG-treated rats. As shown in Figure 2A, the anti-Fx1A binding to the glomerulus was equal in control and IgG-treated rats. Quantification by confocal microscopy demonstrated 2770 ± 93 average fluorescence units per area in control and 2929 ± 136 in IgG-treated rats. Therefore, the beneficial effect of IgG did not result from decreased pathogenic antibody deposition.

We then analyzed deposition of complement components in the glomerulus. We have previously shown that C3c staining is an indicator of ongoing complement attack because it is transient and detectable only during active complement activation and deposition in this model [38]. Staining for C3c was significantly decreased in the IgG-treated group compared with controls (1569 ± 220 fluorescence units per area vs. 2171 ± 526, $P < 0.05$; Fig. 2B). These data are consistent with the hypothesis that SIG administration caused a reduction in ongoing complement deposition.

To confirm this hypothesis, we performed quantitative immunofluorescence study of glomerular C5b-9 staining. Staining for C5b-9 was also significantly decreased in the IgG treated group compared with controls (1201 ± 360 fluorescence units per area vs. 1838 ± 395, $P < 0.05$; Fig. 2C).

Electron microscopy

Ultrastructural examination was performed on biopsies from four animals (2 representative animals from the vehicle treated control group, and 2 representative animals from the SIG treated group). Each animal examined demonstrated numerous, discrete electron dense deposits confined to subepithelial portions of the peripheral glomerular capillary walls (Fig. 3). No differences in amount, size, distribution, or granular appearance of these deposits were detectable amongst any of the animals examined. Foot process effacement in pedicles overlying these electron dense deposits was uniform in all animals.

There was a trend towards better preservation of individual foot processes in those portions of the glomerular capillaries without immune deposits in the animals with lesser degrees of proteinuria, but this trend was not uniform amongst all capillary loops examined. No other abnormalities of glomerular, tubular, or interstitial structure were identified in animals of either group.

Urinary C5b-9 measurement

We have previously shown that urinary C5b-9 excretion reflects active glomerular immune deposit formation in PHN [32]. To confirm attenuation of complement mediated glomerular injury by SIG administration, we measured urinary C5b-9 excretion of both control and IgG-treated animals. IgG-treated rats demonstrated lower C5b-9 excretion compared with control animals with a marginal significance (2.23 ± 0.22 units/24 hr vs. 4.54 ± 2.02, $P = 0.06$).

Complement status in treated rats

To examine if SIG affected systemic complement levels, we measured serum C3 and CH50 levels throughout the experimental period. As shown in Figure 4, there was no evidence of systemic complement activation and no significant difference between control and IgG-treated rats.

Renal function in treated rats

The deterioration of renal function in SIG treated rats might cause the reduction of the total protein excreted into urine. To

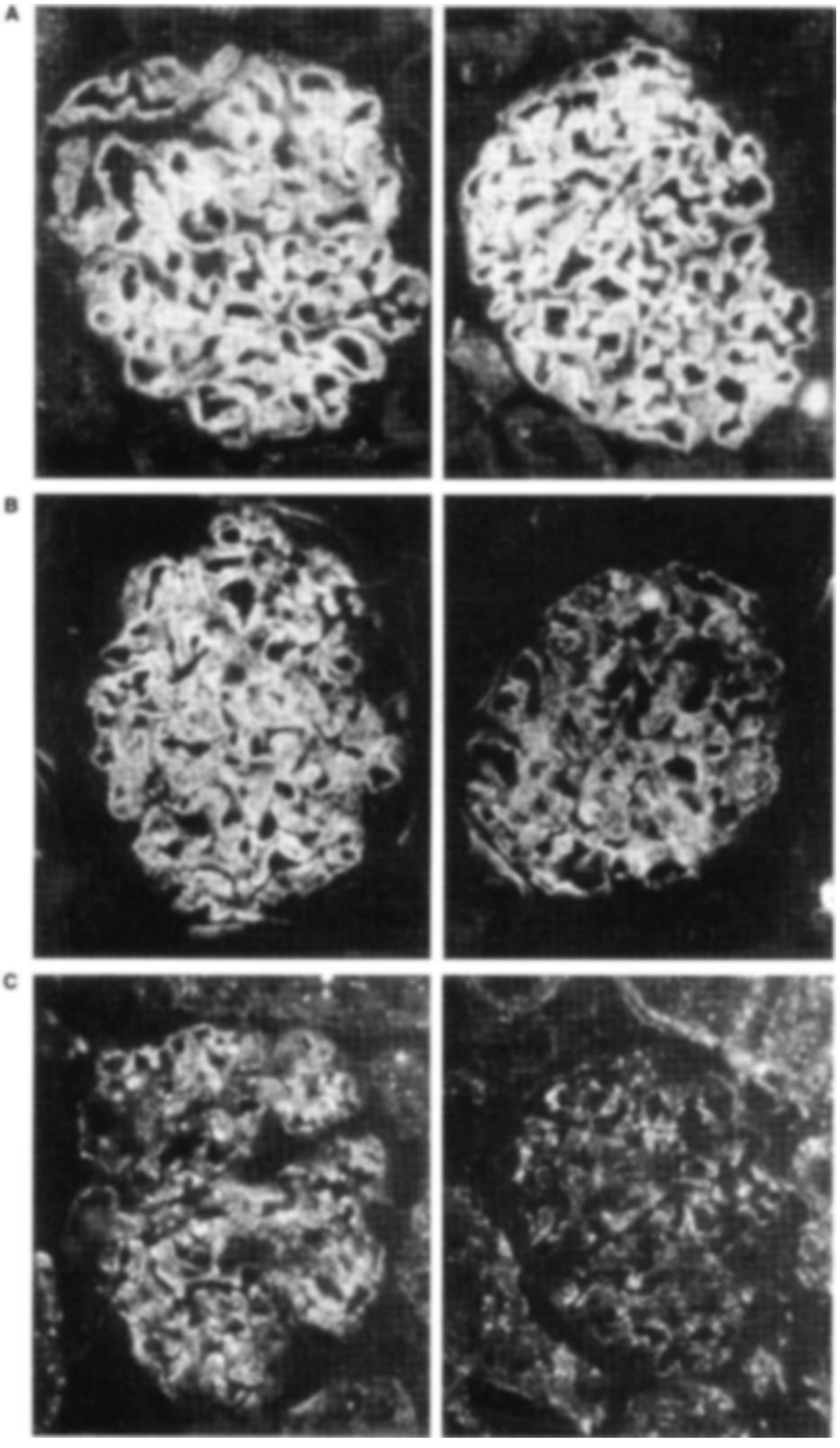

Fig. 2. *Immunofluorescent photographs of representative glomeruli in control (left panels) and IgG treated (right panels) rats with PHN at day 5.* Tissues were stained for sheep IgG (**A**), rat C3c (**B**), and C5b-9 (**C**). Deposition of IgG is similar in both groups, but C3c and C5b-9 staining is reduced in the IgG treated animal (magnification ×40).

confirm that intact function was similar in both groups, we measured BUN at day 5. There was no significant difference in BUN levels between IgG-treated and control rats (23.7 ± 4.9 mg/dl vs. 20.4 ± 3.0, $P > 0.05$).

In vitro *studies*

To confirm that goat IgG can attenuate anti-Fx1A antibody and complement-induced GEC injury, we performed complement-mediated cell lysis assays utilizing cultured GEC. Cultured GEC

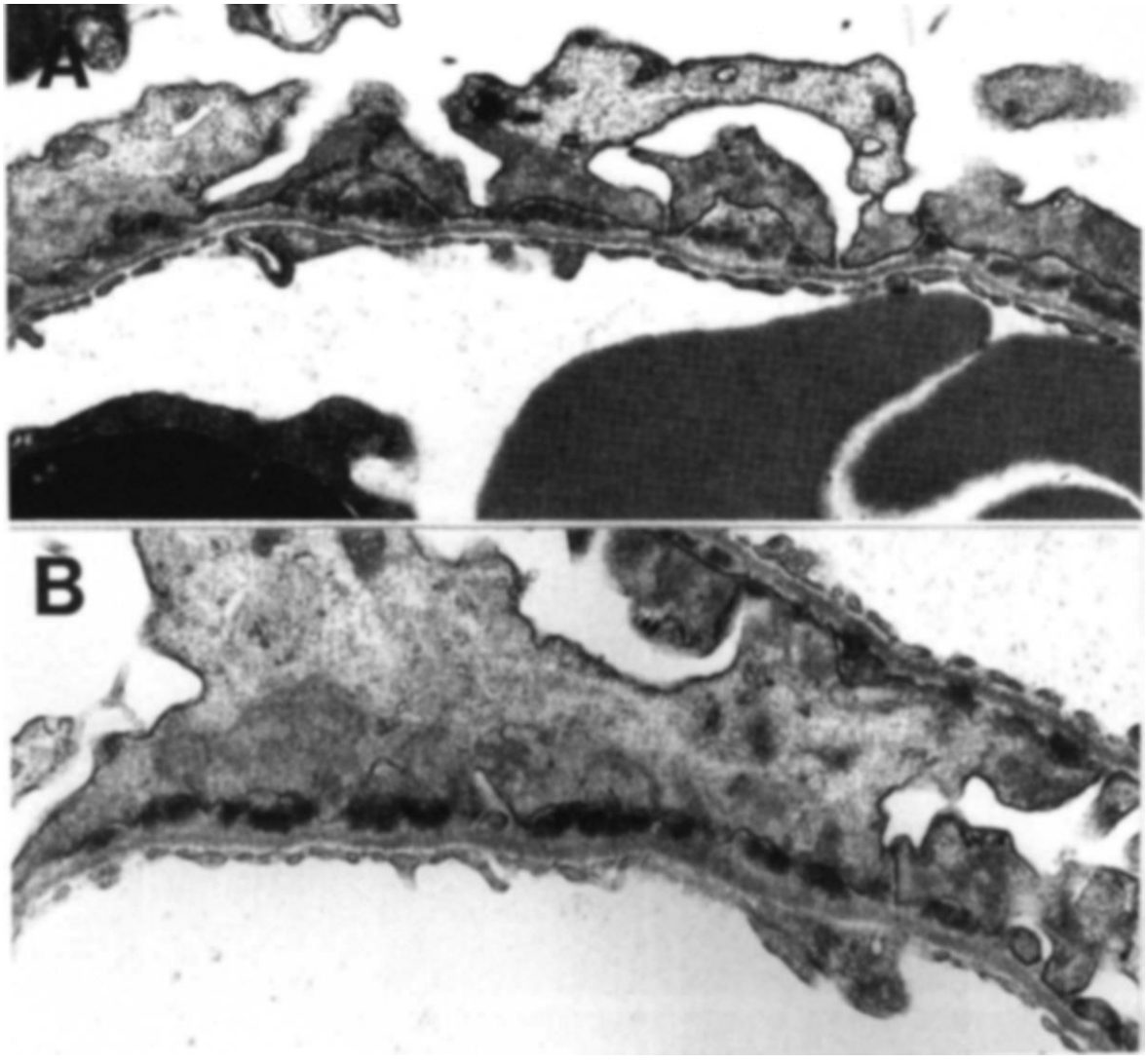

Fig. 3. *Ultrastructural appearance of glomeruli from control rats (***A***) and SIG treated rats (***B***) (magnification ×17750).* Numerous subepithelial immune deposits are present in the capillary walls of both, with similar effacement of epithelial cell foot processes overlying the deposits.

were sensitized with 3 mg/ml anti-Fx1A, followed by incubation with 10% of normal rat serum as the complement source. Complement-induced GEC lysis was expressed as a % of total cell lysis achieved with Triton X-100. At 10 mg/ml goat IgG, cell lysis occurred without statistically significant difference in both cells treated with IgG and controls (37.0 ± 12.9% and 50.5 ± 5.0%, respectively, $N = 4$). However, when 20 mg/ml goat IgG was added, cell lysis was significantly inhibited in the IgG-treated group (16.5 ± 10.6%, $N = 4$, $P < 0.01$; Fig. 5).

We also investigated the effects of homologous IgG on complement-induced GEC injury. Purity of the rat IgG preparation was confirmed by coomassie brilliant blue staining of SDS-PAGE (Fig. 1). Purified rat IgG demonstrated a remarkably protective effects on GEC at 10 mg/ml compared with cells treated with 5 mg/ml of IgG and controls (4.8 ± 6.2%, 31.8 ± 6.3%, and 47.2 ± 11.2%, respectively, $N = 5$, $P < 0.01$; Fig. 5).

Discussion

Since the initial observation in 1981 that patients with idiopathic thrombocytopenic purpura showed a marked improvement in response to high-dose IVIG [45], IVIG has been used as a therapeutic agent in the treatment of some autoimmune diseases including thrombocytopenic purpura, Kawasaki disease, and myasthenia gravis. The therapeutic effects of IVIG have been attributed to various mechanisms including the blocking of Fc receptors and anti-idiotype inhibition of pathogenic Ig [25–27, 46, 47]. IVIG has also been reported to be effective in various renal diseases, including lupus nephritis [22], antineutrophil cytoplasmic antibody nephritis [21], hemolytic uremic syndrome [48], type II membranoproliferative glomerulonephritis [49], and severe IgA nephropathy [23]. In addition, preliminary trials of IVIG in patients with idiopathic MN have shown some benefit. The authors speculated that the mechanisms of action of IgG might be dissociation of subepithelial immune complex deposits or inhibition of an anti-idiotypic antibody response [24].

However, in addition to the possible beneficial effects of IVIG described above, it has recently been pointed out that Ig may also regulate the complement cascade by preventing the binding of activated C3 to antibody-coated targets [30, 31]. In both MN and

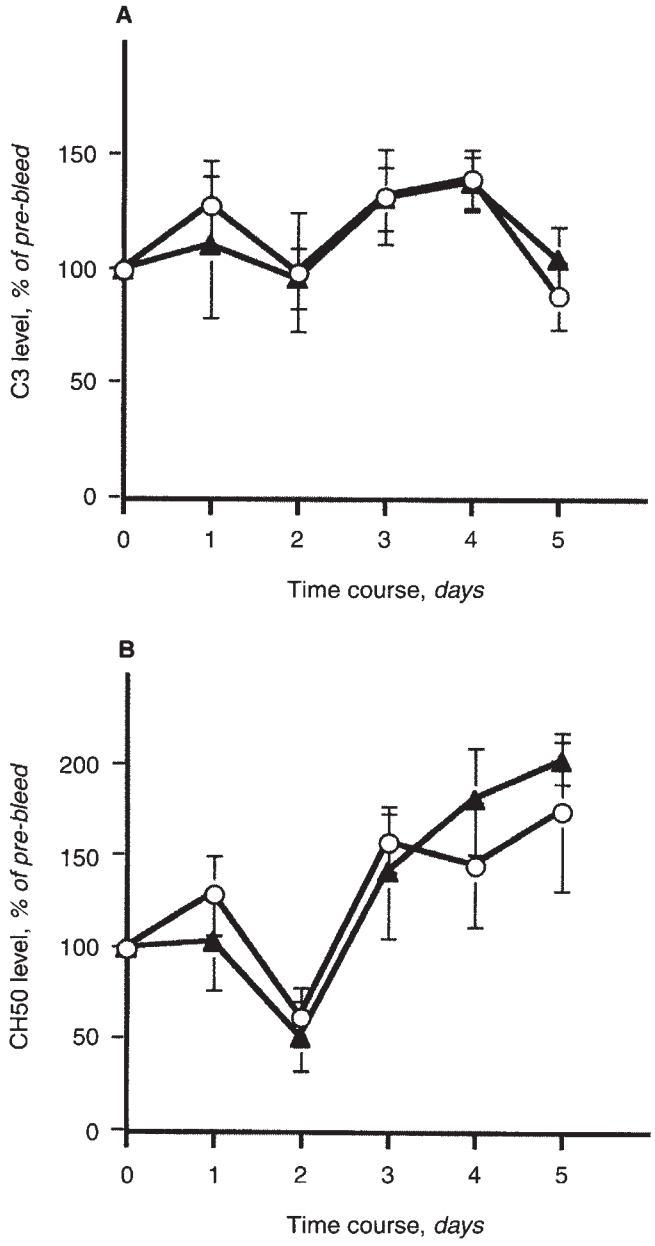

Fig. 4. *Systemic complement status of the rats.* No significant differences were detected in serum C3 (**A**) and CH50 (**B**) levels between control (○) and IgG-treated (▲) rats.

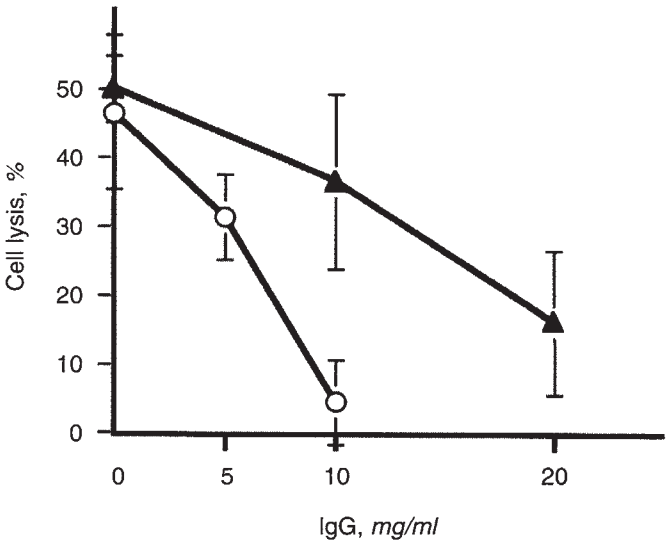

Fig. 5. *Effects of IgG on complement mediated cell lysis of cultured GEC.* Cultured GEC sensitized with anti-Fx1A were incubated with rat complement. A total of 20 mg/ml of goat IgG (▲) inhibited complement-mediated cell lysis of cultured GEC. Furthermore, homologous rat IgG (○) showed much stronger inhibitory effect on GEC lysis even at a lower concentration.

IgA nephropathy treated with IVIG, decreased C3 deposition in glomeruli has been observed [23, 24]. Here we report improvement of PHN by SIG through an apparent complement modifying mechanism. The dose of IgG we injected (600 mg/kg) was equivalent to that used in therapeutic studies in humans (400 to 2000 mg/kg) [25]. The intraperitoneal route was chosen for injection because the volume of IgG solution was too great for intravenous injection. It has previously been shown that levels of IgG in the plasma of rodents are similar when IgG is administered by intravenous or intraperitoneal routes [50]. SIG treatment did not alter glomerular antibody deposition but did reduce proteinuria by over 50%. This was accompanied by reduction in glomerular staining for C5b-9 and C3c, an index of ongoing complement activation, and reduced urinary C5b-9 excretion.

Complement activation mediates immune glomerular injury through several mechanisms including localization of circulating inflammatory cells through generation of chemotactic factors and immune adherence mechanisms and direct injury to resident glomerular cells through membrane insertion of the membrane attack complex, C5b-9 [51, 52]. The role of C5b-9 in mediating changes in glomerular permeability has been particularly well established in models of MN induced by passive administration of antibody to antigens on the membrane of GEC (PHN) [20, 33, 53].

SIG might attenuate complement-mediated tissue injury through several mechanisms. First, the IgG preparation by itself might activate and deplete complement. This mechanism is unlikely in our study because measurement of circulatory C3 and CH50 demonstrated no difference between control and IgG-treated animals. Second, interference with the deposition of anti-Fx1A in glomeruli might also occur by accelerating antibody clearance or reducing glomerular delivery. However, our quantitative immunohistochemical analysis and electron microscopic study demonstrated no differences in antibody deposition or immune complex formation between SIG treated and control animals. Third, immunoglobulin can prevent complement activation by binding to C3 and C4 to prevent its interaction with cell membranes. Our observation of a decrease in C3c deposition without any change in the plasma level of C3, a finding also reported by others [30], supports this mechanism.

IgG is known to be an efficient acceptor for C3 and C4 fragments including C3b [54–57]. High levels of fluid-phase IgG have been shown to compete directly for nascent C3b [58].

Therefore supraphysiologic amounts of IgG may divert C3b from target membranes and suppress complement-mediated disease. High-dose immunoglobulin has been shown to have a beneficial effect on the Forssman shock model [31] and xenotransplantation [28, 29] by modulating complement-mediated tissue injury. We believe that systemic immunoglobulin therapy reduced proteinuria in PHN in our study by the same complement modifying mechanism.

Although we did not completely abolish urinary protein excretion with systemic immunoglobulin as is usually achieved with complement depletion with cobra venom factor [59], the reduction in proteinuria (52%) was greater than that achieved by recombinant complement receptor 1 treatment in the same model (37% reduction) [60].

Our hypothesis that the reduction in proteinuria in SIG treated group was mediated by modifying the effect of complement induced injury is supported by the finding that the glomerular deposition of C3c was decreased in SIG treated rats. Activation of C3 occurs as the result of proteolysis. The cleavage produces a small polypeptide, anaphylatoxin C3a, and activated C3b, which binds to the target surface and initiates C5b-9 assembly. C3b is further degraded into "c" and "d" fragments. The C3c fragment is rapidly released from the binding site, whereas C3d remains covalently attached to target membranes [61–64]. Utilizing these characteristics of C3c and C3d, we have previously shown that positive staining for C3c documents on-going complement activation in the glomerulus, while C3d deposits persist despite lack of ongoing complement activity and do not closely parallel disease activity [38]. Therefore our quantitative immunohistochemical data suggest that ongoing complement activation in glomeruli is suppressed by SIG treatment. This conclusion is also supported by the decrease in glomerular staining for C5b-9 and the greater than 50% reduction in urinary C5b-9 in SIG treated animals, although the latter result did not reach statistical significance.

The conclusion that IgG can modulate C5b-9 induced GEC injury is further supported by *in vitro* data which document a significant reduction in C5b-9 induced GEC lysis in the presence of IgG. Goat IgG demonstrated protective effects on complement-mediated GEC lysis at the concentration of 20 mg/ml. While this concentration exceeds that achieved *in vivo*, it is likely that the amounts of C5b-9 inserted into GEC *in vivo*, where lysis is usually not seen, is substantially less than that achieved *in vitro* and probably suppressible at lower IgG concentration. Moreover, the pathogenic effect of C5b-9 *in vivo* is believed to reflect sublytic effects of C5b-9 on the GEC leading to production by the cell of inflammatory mediators such as oxidants and proteases [51, 65–69], effects which occur with much smaller amounts of membrane-inserted C5b-9 than those required to produce cell lysis. Moreover, we also found that homologous rat IgG was more effective than goat IgG in protection of GEC from complement-mediated cell lysis *in vitro* with significant protection produced at concentrations of only 10 mg/ml.

In conclusion, SIG significantly ameliorated glomerular injury in PHN as documented by a greater than 50% reduction in urine protein excretion. The reduction in glomerular C3c and C5b-9 deposition as well as urinary C5b-9 excretion suggest that this effect was consequent to a reduction in complement activation by SIG. SIG deserves more formal evaluation as a potential therapeutic tool in complement-mediated immune glomerular disease in humans.

Acknowledgments

Support for these studies was provided by research grants from the United States Public Health Service (DK34198, DK43422, and DK02142) and by a George M. O'Brien Kidney Research Center grant (DK47659). Dr. Nangaku was also supported by a research fellowship award from the Eli Lilly Corporation. We thank Audrey Wass and Steve MacFarlane for assistance with electron microscopy. We also thank Dr. Kiyoshi Kurokawa (1st Department of Internal Medicine, University of Tokyo, Japan) for his kind support.

Reprint requests to Dr. William G. Couser, Division of Nephrology, Box 356521, University of Washington Medical Center, 1959 NE Pacific Avenue, BB-1248 Health Sciences Building, Seattle, Washington 98195-6521, USA.

References

1. Coggins CH, Frommer JP, Glassock RJ: Membranous nephropathy. *Semin Nephrol* 2:264–273, 1982
2. Couser WG: Glomerular diseases, in *Textbook of Medicine. A Systematic Approach*, edited by Stein J, Boston, Little Brown and Co., 1986
3. Mallick MP, Short CD, Manos J: Clinical membranous nephropathy. *Nephron* 34:209–219, 1983
4. Noel LH, Zanetti M, Droz D, Barbanel C: Long-term prognosis of idiopathic membranous glomerulonephritis. Study of 116 untreated patients. *Am J Med* 66:82–90, 1979
5. Row PG, Cameron JS, Turner DR, Evans DJ, White RH, Ogg CS, Chantler C, Brown CB: Membranous nephropathy. Long term follow up and associated with neoplasia. *Q J Med* 45:207–239, 1975
6. System USRD: *USRDS 1994 Annual Data Report.* Bethesda, The National Institute of Health, National Institute of Diabetes and Digestive and Kidney Diseases, 1994
7. Cameron JS: Membranous nephropathy and its treatment. *Nephrol Dial Transplant* 7:S72–S79, 1992
8. Austin IHA, Antonovych TT, MacKay K, Boumpas DT, Balow JE: Membranous nephropathy. NIH conference. *Ann Intern Med* 116:672–682, 1992
9. Remuzzi G, Schieppati A, Garattini S: Treatment of idiopathic membranous glomerulopathy. *Curr Opin Nephrol Hypertens* 3:155–163, 1994
10. Fuiano G, Stanziale P, Balletta M, Sepe V, Marinelli G, Comi N, Esposito A, Andreucci VE: Effectiveness of steroid therapy in different stages of membranous nephropathy. *Nephrol Dial Transplant* 4:1022–1029, 1989
11. Donadio JVJ, Holley KE, Anderson CF, Taylor WF: Controlled trial of cyclophosphamide in idiopathic membranous nephropathy. *Kidney Int* 6:431–439, 1974
12. Cattran DC, Delmore T, Roscoe J, Cole E, Cardella C, Charron R, Ritchie S, Toronto Glomerulonephritis Group: A randomized controlled trial of prednisone in patients with idiopathic membranous nephropathy. *N Engl J Med* 320:210–215, 1989
13. Cameron JS, Healy MJR, Adu D: The Medical Research Council trial of short term high dose alternate day prednisolone in idiopathic membranous nephropathy with a nephrotic syndrome in adults. *Q J Med* 74:133–156, 1990
14. Ponticelli C, Zucchelli P, Imbasciatti E, Cagnoli L, Pozzi C, Passerini P, Grassi C, Limido D, Pasquali S, Volpini T, Sasdelli M, Locatelli F: Controlled trial of methylprednisolone and chlorambucil in idiopathic membranous nephropathy. *N Engl J Med* 310:946–950, 1984
15. Mathieson PW, Turner AN, Maidment CGH, Evans DJ, Rees AJ: Prednisolone and chlorambucil treatment in idiopathic membranous nephropathy with deteriorating renal function. *Lancet* 2:869–872, 1988
16. Couser WG, Abrass CK: Pathogenesis of membranous nephropathy. *Annu Rev Med* 39:517–530, 1988
17. Couser WG: Mediation of immune glomerular injury. *J Am Soc Nephrol* 1:13–29, 1990
18. Couser WG: Pathogenesis of glomerulonephritis. *Kidney Int* 44(Suppl 42):S19–S26, 1993

19. 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
20. SAVIN VJ, JOHNSON RJ, COUSER WG: C5b-9 increases albumin permeability of isolated glomeruli in vitro. *Kidney Int* 46:382–387, 1994
21. JAYNE DR, DAVIES MJ, FOX CJ, BLACK CM, LOCKWOOD CM: Treatment of systemic vasculitis with pooled intravenous immunoglobulin. *Lancet* 337:1137–1139, 1991
22. LIN CY, HSU HC, CHIANG H: Improvement of histological and immunological change in steroid and immunosuppressive drug-resistant lupus nephritis by high-dose intravenous gamma globulin. *Nephron* 53:303–310, 1989
23. ROSTOKER G, DESVAUX-BELGHITI D, PILATTE Y, PETIT-PHAR M, PHILIPPON C, DEFORGES L, TERZIDIS H, INTRATOR L, ANDRE C, ADNOT S, BONIN P, BIERLING P, REMY P, LAGRUE G, LANG P, WEIL B: High-dose immunoglobulin therapy for severe IgA nephropathy and Henoch-Schönlein purpura. *Ann Intern Med* 120:476–484, 1994
24. PALLA R, CIRAMI C, PANICHI V, BIANCHI AM, PARRINI M, GRAZI G: Intravenous immunoglobulin therapy of membranous nephropathy: Efficacy and safety. *Clin Nephrol* 35:98–104, 1991
25. DWYER JM: Manipulating the immune system with immune globulin. *N Engl J Med* 326:107–116, 1992
26. BALLOW M: Mechanisms of action of intravenous immunoglobulin therapy and potential use in autoimmune connective tissue diseases. *Cancer* 68:1430–1436, 1991
27. RONDA N, HUREZ V, KAZATCHKINE MD: Intravenous immunoglobulin therapy of autoimmune and systemic inflammatory diseases. *Vox Sang* 64:65–72, 1993
28. MAGEE JC, COLLINS BH, HARLAND RC, LINDMAN BJ, BOLLINGER RR, FRANK MM, PLATT JL: Immunoglobulin prevents complement-mediated hyperacute rejection in swine-to-primate xenotransplantation. *J Clin Invest* 96:2404–2412, 1995
29. GAUTREAU C, KOJIMA T, WOIMANT G, CARDOSO J, DEVILLIER P, HOUSSIN D: Use of intravenous immunoglobulin to delay xenogeneic hyperacute rejection. *Transplantation* 60:903–907, 1995
30. FRANK MM, BASTA M, FRIES LF: The effects of intravenous immune globulin on complement-dependent immune damage of cells and tissues. *Clin Immunol Immunopathol* 62:S82–86, 1992
31. BASTA M, KIRSHBOM P, FRANK MM, FRIES LF: Mechanism of therapeutic effect on high-dose intravenous immunoglobulin: Attenuation of acute, complement-dependent immune damage in a guinea pig model. *J Clin Invest* 84:1974–1981, 1989
32. SCHULZE M, BAKER PJ, PERKINSON DT, JOHNSON RJ, OCHI RF, STAHL RA, COUSER WG: Increased urinary excretion of C5b-9 distinguishes passive Heymann nephritis in the rat. *Kidney Int* 35:60–68, 1989
33. 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* 135:185–194, 1989
34. BRADLEY GM, BENSON ES: Examination of the urine, Todd-Stanford, in *Clinical Diagnosis by Laboratory Methods* (15th ed), edited by DAVIDSON I, HENRY JB, Philadelphia, WB Saunders, 1974
35. RUSSO C, CALLEGARO L, LANZA E, FERRONE S: Purification of IgG monoclonal antibody by caprylic acid precipitation. *J Immunol Meth* 65:269–271, 1983
36. SCHWARTZ RS: Overview of the biochemistry and safety of a new native intravenous gamma globulin, IGIV, pH 4.25. *Am J Med* 83(Suppl 4A):46–51, 1987
37. HEREMANS JF: Antigen titration by simple radial immunodiffusion in plates, in *Methods in Immunology and Immunochemistry* (vol III), edited by WILLIAMS CA, CHASE MW, New York, Academic Press, 1971
38. SCHULZE M, PRUCHNO CJ, BURNS M, BAKER PJ, JOHNSON RJ, COUSER WG: Glomerular C3c localization indicates ongoing immune deposit formation and complement activation in experimental glomerulonephritis. *Am J Pathol* 142:179–187, 1993
39. BRANDT J, PIPPIN J, SCHULZE M, HÄNSCH GM, ALPERS CE, JOHNSON RJ, GORDON K, COUSER WG: Role of the complement membrane attack complex (C5b-9) in mediating experimental mesangioproliferative glomerulonephritis. *Kidney Int* 49:335–343, 1996
40. NELSON RA, JENSEN J, GIGLI I, TAMURA N: Methods for the separation, purification and measurement of nine components of hemolytic complement in guinea-pig serum. *Immunochemistry* 3:111–135, 1966
41. PRUCHNO CJ, BURNS M, SCHULZE M, JOHNSON RJ, BAKER PJ, ALPERS CE, COUSER WG: Urinary excretion of the C5b-9 membrane attack complex of complement is a marker of immune disease activity in autologous immune complex nephritis. *Am J Pathol* 138:203–211, 1991
42. JOHNSON RJ, YAMABE H, CHEN YP, CAMPBELL C, GORDON K, BAKER P, LOVETT D, COUSER WG: Glomerular epithelial cells secrete a glomerular basement membrane-degrading metalloproteinase. *J Am Soc Nephrol* 2:1388–1397, 1992
43. RICHARDSON CA, GORDON KL, COUSER WG, BOMSZTYK K: IL-1β increases laminin B2 chain mRNA levels and activates NF-κB in rat glomerular epithelial cells. *Am J Physiol* 268:F273–F278, 1995
44. FLOEGE J, JOHNSON RJ, GORDON K, YOSHIMURA A, CAMPBELL C, IRUELA-ARISPE L, ALPERS CE, COUSER WG: Altered glomerular extracellular matrix synthesis in experimental membranous nephropathy. *Kidney Int* 42:573–585, 1992
45. IMBACH P, BARANDUN S, D'APUZZO V, BAUMGARTNER C, HIRT A, MORELL A, ROSSI E, SCHONI M, VEST M, WAGNER HP: High-dose intravenous gammaglobulin for idiopathic thrombocytopenic purpura in childhood. *Lancet* 1:1228–1231, 1981
46. JORDAN SC, TOYODA M: Treatment of autoimmune diseases and systemic vasculitis with pooled human intravenous immune globulin. *Clin Exp Immunol* 97(Suppl 1):31–38, 1994
47. SMILEY JD, TALBERT MG: High-dose intravenous gamma globulin therapy: How does it work? *Am J Med Sci* 309:295–303, 1995
48. REMUZZI G: HUS and TTP: Variable expression of a single entity. *Kidney Int* 32:292–308, 1987
49. MIYAKAWA S, UENO T, MOCHIZUKI H, ISHIDOYA N, TAHARA H, MURAMATSU Y, FUJISAWA Y, USUI N, AKATSUKA J, AIZAWA S: High dose intravenous gammaglobulin therapy against pediatric intractable renal diseases. *Jikeikai Med J* 33:241–252, 1986
50. EMERSON JTE, COLLINS MS, BUDINGER MD: Use of a new, low-pH immunoglobulin G preparation during episodes of bacteremia in the rat. *Rev Infect Dis* 8(Suppl 4):S409–S419, 1986
51. COUSER WG, SCHULZE M, PRUCHNO CJ: Role of C5b-9 in experimental membranous nephropathy. *Nephrol Dial Transplant* 7:S25–S31, 1992
52. HEBERT LA, COSIO FG, BIRMINGHAM DJ: The role of complement system in renal injury. *Semin Nephrol* 12:408–427, 1992
53. CYBULSKY AV, RENNKE HG, FEINTZEIG ID, SALANT DJ: Complement-induced glomerular epithelial cell injury. Role of the membrane attack complex in rat membranous nephropathy. *J Clin Invest* 77: 1096–1107, 1986
54. FRIES LF, GAITHER TA, HAMMER CH, FRANK MM: C3b covalently bound to IgG demonstrates a reduced rate of inactivation by factor H and I. *J Exp Med* 160:1640–1655, 1984
55. GADD KJ, REID KBM: The binding of complement component C3 to antibody-antigen aggregates after activation of the alternative pathway in human serum. *Biochem J* 195:471–480, 1981
56. BROWN E, BERGER JM, JOINER KA, FRANK MM: Classical complement pathway activation by antipneumococcal antibodies leads to covalent binding of C3b to antibody molecules. *Infect Immun* 42:594–598, 1983
57. JACOBS RJ, REICHLIN M: C3-bearing immunoglobulin in human serum. *J Immunol* 130:2775–2781, 1983
58. BERGER M, ROSENKRANZ P, BROWN CY: Intravenous and standard immune serum globulin preparations interfere with uptake of ^{125}I-C3 onto sensitized erythrocytes and inhibit hemolytic complement activity. *Clin Immunol Immunopathol* 34:227–236, 1985
59. SALANT DJ, BELOK S, MADAIO MP, COUSER WG: A new role for complement in experimental membranous nephropathy in rats. *J Clin Invest* 66:1339–1350, 1980
60. COUSER WG, JOHNSON RJ, YOUNG BA, YEH CG, TOTH CA, RUDOLPH AR: The effects of soluble recombinant complement receptor 1 on complement-mediated experimental glomerulonephritis. *J Am Soc Nephrol* 5:1888–1894, 1995
61. TACK BF: The β-Cys-γ-Glu thioester bond in human C3. C4, and α2-macroglobulin. *Springer Semin Immunopathol* 6:259–282, 1983
62. PANGBURN MK, MULLER-EBERHARDT HJ: The C3 convertase of the

alternative pathway of human complement. Enzymatic properties of the biomolecular proteinase. *Biochem J* 235:723–730, 1986
63. MULLER-EBERHARDT HJ: Complement: Chemistry and pathways, in *Inflammation: Basic Principles and Clinical Correlates*, edited by GALLIN JI, GOLDSTEIN IM, SYNDERMAN R, New York, Raven Press, 1988
64. MOLLNES TE, LACHMANN PJ: Regulation of complement. *Scand J Immunol* 27:127–142, 1988
65. HÄNSCH GM, BETZ M, GUNTHER J, ROTHER KO, STERZEL B: The complement membrane attack complex stimulates the prostanoid production of cultured glomerular epithelial cells. *Int Arch Allergy Appl Immunol* 85:87–93, 1988
66. CYBULSKY AV, SALANT DJ, QUIGG RJ, BADALAMENTI J, BONVENTRE JV: Complement C5b-9 complex activates phospholipases in glomerular epithelial cells. *Am J Physiol* 257:F826–F836, 1989
67. CYBULSKY AV, BONVENTRE JV, QUIGG RJ, LIEBERTHAL W, SALANT DJ: Cytosolic calcium and protein kinase C reduce complement-mediated glomerular epithelial cell injury. *Kidney Int* 38:803–811, 1990
68. WAGNER C, BRAUNGER M, BEER M, ROTHER K, HÄNSCH GM: Induction of matrix protein synthesis in human glomerular mesangial cells by the terminal complement complex. *Exp Nephrol* 2:51–56, 1994
69. QUIGG RJ, CYBULSKY AV, JACOBS JB, SALANT DJ: Anti-Fx1a produces complement-dependent cytotoxicity of glomerular epithelial cells. *Kidney Int* 34:43–52, 1988