C5b-9 membrane attack complex mediates endothelial cell apoptosis in experimental glomerulonephritis

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Hughes, Jeremy, Masaomi Nangaku, Charles E. Alpers, Stuart J. Shankland, William G. Couser, and Richard J. Johnson. C5b-9 membrane attack complex mediates endothelial cell apoptosis in experimental glomerulonephritis. Am J Physiol Renal Physiol 278: F747–F757, 2000.—We studied the role of the C5b-9 membrane attack complex in two models of inflammatory glomerulonephritis (GN) initiated by acute glomerular endothelial injury in Piebald-viral-Glaxo (PVG) complement-sufficient rats, C6-deficient rats (C6−), and rats systematically depleted of complement with cobra venom factor (CVF). GN was induced by performing a left nephrectomy and selectively perfusing the right kidney with either 1) the lectin concanavalin A (Con A) followed by complement-fixing anti-Con A (Con A GN) or 2) purified complement-fixing goat anti-glomerular endothelial cell (GEN) antibody [immune-mediated thrombotic microangiopathy (ITM)]. Comparable levels of GEN apoptosis were detected in C+ animals in both models. CVF administration reduced GEN apoptosis by 10- to 12-fold. GEN apoptosis was C5b-9 dependent because PVG C6− rats were protected from GEN loss. Furthermore, functional inhibition of the cell surface complement regulatory protein CD59 by renal perfusion with anti-CD59 antibody in ITM resulted in a 3.5-fold increase in GEN apoptosis. Last, in Con A GN, abrogation of GEN apoptosis preserved endothelial integrity and renal function. This study demonstrates the specific role of C5b-9 in the induction of GEN apoptosis in experimental inflammatory GN, a finding with implications for diseases associated with the presence of antiendothelial cell antibodies.

complement; inflammation; cell death

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The complement system is an important innate humoral defense system comprised of ~20 plasma proteins that may be activated in a cascade fashion by either the classic pathway (immune complex mediated) or the alternative pathway (injured cells, microbes, etc.). A regulatory system of both plasma proteins (e.g., factor H, factor I, factor S, clusterin) and membrane-bound proteins (e.g., CD59, decay accelerating factor [DAF], and membrane cofactor protein [MCP]) act to prevent the inappropriate activation of complement by autologous cells (reviewed in Ref. 49). Complement activation leads to the generation of multiple C5b-9 membrane attack complexes that insert into the cell membrane. The C5b-9 membrane attack complex has been unequivocally demonstrated to be involved in the development of proteinuria in experimental membranous nephropathy (passive Heymann nephritis), mesangial cell injury in the Thy 1.1 model of mesangioproliferative glomerulonephritis, and glomerular endothelial cell injury in a model of immune-mediated thrombotic microangiopathy (4, 11, 47). In addition, renal injury may be modulated by manipulation of complement regulatory proteins such that overexpression of CD59 protects mesangial cells from complement-mediated injury in vitro whereas the administration of exo-
genous, soluble recombinant complement receptor 1 (CR1) results in significant amelioration of renal disease in vivo (10, 51).

In the extrapolation from the well-known lytic effect of complement on nonnucleated erythrocytes, it has, until recently, been thought that the insertion of multiple C5b-9 membrane attack complexes into the cell membrane of a nucleated cell would inevitably lead to cell death by necrosis (54). However, it has become apparent that cells have various responses to attack by complement. For example, sublytic complement attack on cells can lead to cellular activation rather than cell death and induce DNA synthesis and cell proliferation (57, 64). The C5b-9 membrane attack complex has also been recently reported to mediate mesangial cell apoptosis in experimental glomerulonephritis (59).

In this study we examined the role of the C5b-9 membrane attack complex in the induction of glomerular endothelial cell apoptosis by utilizing two models, the concanavalin A model of immune complex glomerulonephritis and a model of thrombotic microangiopathy, both initiated by injury to the glomerular endothelium (31, 46). We separated the role of the C5b-9 membrane attack complex from earlier complement components by examining the effects of cobra venom factor (which depletes all complement components and prevents the generation of the anaphylotoxins C3a and C5a, as well C5b-9, etc.) and isolated C6 deficiency by using C6-deficient PVG rats, which can generate C3a and C5a but are unable to form the C5b-9 membrane attack complex (71). Our results indicate that complement is the principal inducer of endothelial cell apoptosis in antibody-mediated glomerulonephritis and that this effect is mediated primarily by sublytic C5b-9.

METHODS

Experimental Animals

Male PVG rats weighing 200–250 g were obtained from two separate vendors. PVG rats with normal complement activity were obtained from Harlan Sprague Dawley (Indianapolis, IN). Age- and sex-matched C6-deficient PVG rats were obtained from Bantin and Kingman Universal (Edmonds, WA). All rats were assessed for complement activity by CH50 assay before the study. Complement component analysis in PVG rats has been reported previously (4, 38).

Experimental Models of Glomerular Endothelial Injury

Concanavalin A (Con A) glomerulonephritis. Glomerular capillary endothelial cell injury was induced in PVG rats by perfusing a left nephrectomy and selectively perfusing the right renal artery with Con A (ICN Biomedicals, Costa Mesa, CA) followed by polyclonal anti-Con A antibody as previously described (31). The lectin Con A binds to sugar residues on glomerular capillary endothelial cells and glomerular basement membrane glycoproteins. The subsequent perfusion of anti-Con A antibody results in antibody binding to this “planted antigen,” with consequent in situ immune complex deposition and complement activation. Schemata was time was <6 min in all cases.

Disease was induced in three groups of rats: normal, complement-sufficient PVG rats (C+/PVG, n = 4); normal PVG rats systemically depleted of complement with cobra venom factor treatment (CVF/PVG, n = 7); and C6-deficient PVG rats (C–/PVG, n = 8). To systemically deplete rats, 125 µg of CVF (Calbiochem, La Jolla, CA) were administered intraperitoneally 1 day before the induction of glomerulonephritis. Rats in other groups received vehicle only instead of CVF. Complement depletion in CVF-treated rats and complement deficiency in C6-deficient rats were confirmed by measurements of CH50. Tail vein blood samples were collected at day –1, just before induction of disease, and at day 1 to examine the complement status. One hour after surgery a biopsy was performed, whereas 24 h after induction of disease a blood sample was obtained via cardiac puncture, and the kidney was removed for histological studies. All studies were performed in an accredited animal care facility in accord with the National Institutes of Health guidelines for the Care and Use of Laboratory Animals. This animal model was approved by the University of Washington Animal Care Committee.

Immune-mediated thrombotic microangiopathy. To confirm the observations in Con A glomerulonephritis studies were also carried out in a second model of antibody-mediated endothelial cell injury induced by anti-endothelial cell antibodies. The characteristics of this model have been previously described (46–48). Briefly, glomerular endothelial cell injury was induced in PVG rats by selective perfusion of the right kidney through the superior mesenteric artery with either 80 mg/kg body wt of purified goat anti-rat glomerular endothelial cell antibody or control goat IgG in PBS, pH 7.2, as previously described (46). Disease was induced in the same three groups of rats studied with Con A: normal, complement-sufficient PVG rats (C+/PVG, n = 6); normal PVG rats systemically depleted of complement with CVF treatment as outlined previously (CVF/PVG, n = 6); and C6-deficient PVG rats (C–/PVG, n = 6). To confirm a role for C5b-9, we also examined the effect of enhancing C5b-9 deposition by blocking the function of the complement regulatory protein CD59. In experiments involving the inhibition of CD59 the kidneys were initially perfused with PBS followed by 0.15 mg of F(ab′)2 fragments of murine anti-CD59 monoclonal antibody 6D1 (generously supplied by Dr. B. P. Morgan, Univ. of Wales, College of Medicine, Cardiff, UK) or control mouse IgG (n = 6 in each group). After being flushed with PBS, the kidney was perfused with 13 mg/kg body wt of purified goat anti-rat glomerular endothelial cell IgG. A reduced amount of anti-rat glomerular endothelial cell IgG was used in these experiments because CD59 blockade was predicted to worsen renal injury. Four hours after surgery a biopsy was performed, whereas 24 h after induction of disease the kidney was removed for histological studies. These studies were performed in an accredited animal care facility in accord with the National Institutes of Health guidelines for the Care and Use of Laboratory Animals.

Renal Histology

Renal biopsies were fixed in methyl Carnoy’s solution and embedded in paraffin. Light microscopy was performed on 4-µm sections of tissue stained with periodic acid Schiff (PAS) reagent and counterstained with hematoxylin.

To perform single immunoperoxidase staining, tissue sections were incubated with the following primary and secondary antibodies as indicated. Glomerular macrophages were stained with the monoclonal antibody ED1 (Bioproducts for Science, Indianapolis, IN) at the final concentration of 0.4 µg/ml (15). Glomerular platelet infiltration was assessed by staining with PL1, a murine monoclonal antibody to rat platelets (generously supplied by W. W. Baker, Univ. of Groningen, The Netherlands) (1). Glomerular endothelial cells were stained with rat endothelial cell antibody 1 (RECA-
1), a monoclonal IgG1 antibody specific for endothelial cells (18). Horseradish peroxidase-conjugated avidin D (Vector Laboratories, Burlingame, CA) was used after all biotinylated secondary antibodies at room temperature for 20 min. Black or brown staining was developed by using diaminobenzidine (DAB; Sigma Chemical, St. Louis, MO) with or without nickel as the chromogen, respectively, and counterstained with methyl green. An irrelevant primary antibody of the same isotype was used as a negative control. Positive control tissue included sections from diseased rats that expressed these antigens. Quantitation of the number of neutrophils per glomerular cross section was performed on PAS-stained sections as described previously (28).

The numbers of neutrophils and macrophages per glomerular cross section were counted in a blinded fashion. Fifty randomly selected glomeruli were examined per rat, and the average number of positive cells per glomerular cross section was calculated. Data were expressed as the mean cell number ± SE per glomerular cross section. Glomeruli containing only a minor portion of the tuft (<20 discrete capillary segments) were excluded.

Semiquantitative assessment of immunohistological staining was performed as described previously (27). Briefly, RECA-1 expression was assessed in a blinded fashion by scoring 50 randomly selected glomeruli by using a 0-5 scale as follows: grade 0, absent expression; grade 1, <24% of capillary loops positive; grade 2, 25–49% of capillary loops positive; grade 3, 50–74% of capillary loops positive; grade 4, 75–94% of capillary loops positive; grade 5, >95% of capillary loops positive. PL-1 expression was similarly quantified in a blinded fashion by scoring 50 randomly selected glomeruli by using a scale of 0-5 as follows: grade 0, absent expression; grade 1, <5% of capillary loops positive; grade 2, 6–24% of capillary loops positive; grade 3, 25–49% of capillary loops positive; grade 4, 50–74% of capillary loops positive; grade 5, >75% of capillary loops positive.

Tissue for immunofluorescence was embedded in OCT (Lab-Tek Products, Miles Laboratories, Naperville, IL) and snap frozen in isopentane. Rat C3 and C5b-9 were detected with FITC-conjugated goat anti-rat C3 (Cappel) and biotinylated anti-rat C5b-9 monoclonal antibody 2A1 followed by FITC-streptavidin, respectively.

Detection of Apoptosis

Apoptotic cells were detected by using two well-established and validated methods. First, cells were identified as undergoing apoptosis on PAS-stained sections when they exhibited marked chromatin condensation, which is the classic morphological hallmark of apoptosis (2, 34). Second, apoptotic cells were identified by the terminal deoxynucleotidyl transferase (TdT)-mediated dUTP-biotin nick end labeling (TUNEL) assay as previously described (2, 25). Briefly, 4-µm tissue sections were deparaffinized and rehydrated in ethanol, followed by an antigen retrieval step comprising boiling in 0.01 M sodium citrate buffer for 2 min. Sections were then incubated with proteinase K (6.2 µg/ml; Boehringer Mannheim, Indianapolis, IN) followed by TdT (300 enzyme units/ml; Pharmacia Biotech, Piscataway, NJ) and Bio-14-dATP (0.94 nm; GIBCO-BRL, Grand Island, NY). Biotinylated ATP was detected by using the ABC staining method (Vector Laboratories; following the manufacturer’s protocol). As a positive control, slides were pretreated with DNase (20 Kunitz units/ml; Sigma Biosciences, St Louis, MO). Cells were regarded as TUNEL positive if their nuclei were stained black and displayed a typical apoptotic morphology with chromatin condensation. The number of apoptotic cells in PAS- or TUNEL-stained tissue sections was counted in 50 sequentially selected glomeruli and expressed as the mean cell number ± SE per glomerular cross section.

Electron Microscopy (EM)

Tissue for EM was fixed in half-strength Karnovsky’s solution (1% paraformaldehyde and 1.25% gluteraldehyde in 0.1 M sodium cacodylate buffer, pH 7.0), postfixed with 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 (Phillips export BV, Eindhoven, The Netherlands) electron microscope.

Blood Urea Nitrogen (BUN) Measurement

BUN was determined colorimetrically by using a commercial kit for the measurement of urea nitrogen (Sigma Diagnostics, St. Louis, MO).

Statistical Methods

All values are expressed as means ± SE. Statistical significance (defined as P < 0.05) was evaluated by using the Student’s t-test.

RESULTS

Glomerular endothelial cells undergo apoptosis in Con A glomerulonephritis and immune-mediated thrombotic microangiopathy. The glomeruli of control complement-sufficient rats with glomerulonephritis were positive for C3 and C5b-9 staining, whereas the glomeruli of CVF-treated rats were negative for C3 and C5b-9 (data not shown). The glomeruli of C6-deficient rats with glomerulonephritis were positive for C3 but negative for C5b-9, which is consistent with C6 deficiency (data not shown).

At 1 h after the induction of Con A glomerulonephritis in complement sufficient rats (C+/PVG), significant numbers of apoptotic cells were evident within the capillary lumina. By PAS staining, the cells within the glomerular capillaries exhibited the typical marked nuclear chromatin condensation that is the morphological hallmark of apoptosis and distinguishes apoptotic cells from cells undergoing necrosis (2, 34) (Fig. 1A). By using similar strict morphological criteria in addition to positive nuclear staining, the TUNEL stain can also distinguish between apoptotic and necrotic cells (43). TUNEL-positive apoptotic cells were evident within the capillary lumen 1 h after induction of disease (Fig. 1B). Apoptotic cells could also be demonstrated within the capillary lumen by using EM (Fig. 1C).

Glomerular endothelial cell apoptosis is markedly diminished in C6-deficient and CVF-treated animals with resultant protection of glomerular endothelial integrity. In both Con A glomerulonephritis and immune-mediated thrombotic microangiopathy both CVF treatment and C6 deficiency resulted in an impressive 10- to 25-fold reduction in the numbers of apoptotic cells evident at 1 or 4 h, respectively, by PAS and TUNEL staining (Fig. 2, A and B). In Con A glomerulonephritis this reduction in apoptosis was associated with significant preservation of the glomerular endothelium as assessed by RECA-1 staining compared with complement-sufficient rats (Fig. 3). It should be noted
that in complement-sufficient rats there was also marked loss of the peritubular capillaries adjacent to affected glomeruli (Fig. 3B).

Glomerular endothelial cell apoptosis is increased in immune-mediated thrombotic microangiopathy after inhibition of glomerular CD59 function. Inhibition of the cell surface complement regulatory protein CD59 with a function-blocking antibody just before induction of immune-mediated thrombotic microangiopathy led to a significant 3.5-fold increase in the number of apoptotic cells evident at the 4-h time point by PAS staining (Fig. 4). A significant increase in the number of apoptotic cells was also evident in TUNEL-stained sections (Fig. 4). Inhibition of CD59 function in this model of glomerular endothelial injury has previously been reported to result in increased loss of the glomerular endothelium as assessed by RECA-1 staining compared with control rats treated with irrelevant antibody (48).

Fig. 1. Glomerular endothelial cells undergo apoptosis in concanavalin-A (Con A) glomerulonephritis. Glomerular endothelial cell apoptosis demonstrated at 1-h time point by periodic acid-Schiff (PAS; A), terminal deoxynucleotidyl transferase (TdT)-mediated dUTP-biotin nick end labeling (TUNEL; B), and electron microscopy (C). Note the markedly condensed nuclear chromatin, which is the classic morphological hallmark of apoptosis.

Fig. 2. Glomerular endothelial cell apoptosis in Con A glomerulonephritis and immune-mediated thrombotic microangiopathy is markedly diminished in C6-deficient (C6−) and cobra venom factor (CVF)-treated animals. CVF treatment and C6 deficiency protect glomerular endothelial cells from undergoing apoptosis in both Con A glomerulonephritis (1-h time point; A) and immune-mediated thrombotic microangiopathy (4-h time point; B). *P < 0.001.
In Con A glomerulonephritis, neutrophil and platelet recruitment at 1 h are reduced by CVF treatment but are unaffected by C6 deficiency. Rapid infiltration of glomeruli with neutrophils and platelets is characteristic of Con A glomerulonephritis (30). CVF treatment markedly diminished glomerular neutrophil infiltration presumably via the reduced production of anaphylatoxins. C6-deficient rats, however, developed a significant glomerular neutrophil infiltrate that was comparable to that evident in complement-sufficient rats (Fig. 5A). A similar result was seen when glomerular platelet infiltration was assessed. CVF treatment almost completely abolished glomerular platelet recruitment, but no significant difference was evident between C6-deficient and complement-sufficient rats (Fig. 5B).

In Con A glomerulonephritis, glomerular macrophage infiltration at 24 h is unaffected by CVF treatment but is significantly increased in the presence of C6 deficiency. CVF treatment had no effect on glomerular macrophage infiltration at 24 h, indicating that early macrophage recruitment in this model is complement independent. C6-deficient rats, however, developed a significant 2.4-fold increase in glomerular ED1-positive macrophage infiltration compared with that seen in complement-sufficient control rats (Fig. 6).

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In Con A glomerulonephritis, C6 deficiency and CVF treatment are associated with preservation of renal function. Both Con A glomerulonephritis and immune-mediated thrombotic microangiopathy induce severe acute renal failure in complement-sufficient rats. In Con A glomerulonephritis, both CVF treatment and C6 deficiency were associated with marked improvement in renal failure as measured by BUN (Fig. 7). In immune-mediated thrombotic microangiopathy, CVF treatment and C6 deficiency have previously been reported to result in significant protection from renal failure (47).

DISCUSSION

The complement cascade and apoptosis play an important role in the pathogenesis of renal injury and the
The proteins involved in the complement cascade and apoptosis are well conserved throughout species, and recent data indicate that complement activation and apoptosis occur together in various biological scenarios. For example, cells undergoing apoptosis exhibit reduced levels of cell surface complement regulatory proteins such as DAF, MCP, and CD59 together with the de novo expression of phosphatidylinerine and other as yet unidentified activators of complement (32, 44). Complement activation by apoptotic cells results in the deposition of opsonizing iC3b on the cell surface, which may play a role in the clearance of apoptotic cells by phagocytes expressing complement receptors (44, 68). Furthermore, complement has been shown to induce myocardial cell apoptosis in vivo after myocardial ische-

Fig. 5. Neutrophil and platelet recruitment at 1 h is reduced by CVF treatment but not C6 deficiency. CVF treatment significantly abrogates neutrophil (A) and platelet (B) recruitment at 1 h, whereas C6 deficiency has no effect (*P < 0.001).

Fig. 6. Glomerular macrophage infiltration at 24 h is unaffected by CVF treatment but is significantly increased by C6 deficiency. CVF treatment has no effect on macrophage infiltration, which is significantly increased in C6-deficient rats with Con A glomerulonephritis (*P < 0.005).

Fig. 7. Renal function is preserved in C6-deficient and CVF-treated animals in Con A glomerulonephritis. Both CVF treatment and C6 deficiency resulted in significant improvement in renal function assessed by BUN 24 h following the induction of Con A glomerulonephritis (*P < 0.005).
mias-reperfusion injury, a model in which anti-C5 antibodies provide protection from cell death (69). Last, a recent report provides convincing data that the C5b-9 membrane attack complex mediates mesangial cell apoptosis in the Thy 1.1 model of glomerulonephritis in the rat (59).

The first major finding of our study is that glomerular endothelial cells undergo apoptosis as the preferred mode of death in two models of renal injury induced by complement fixing antibodies: Con A glomerulonephritis and immune-mediated thrombotic microangiopathy. We identified apoptotic cells in PAS- and TUNEL-stained tissue sections by using strict morphological criteria that have been validated in previous studies and also shown to distinguish apoptotic cells from those undergoing necrosis (2, 34, 43). There are several lines of evidence indicating that the apoptotic cells evident in complement-sufficient rats with Con A glomerulonephritis are very likely to be derived from the targeted glomerular endothelium in this model. First, the presence of significant numbers of apoptotic cells in complement-sufficient animals was associated with the significant loss of glomerular endothelial cells as assessed by staining with the endothelium-specific antibody RECA-1. In contrast, the profound reduction of apoptosis evident in complement-depleted or C6-deficient rats was associated with the preservation of an almost intact endothelium. We obviously considered the possibility that a significant proportion of the apoptotic cells present within the glomerular capillaries were derived from infiltrating neutrophils, which are recruited very early in the course of disease in Con A glomerulonephritis and are known to undergo constitutive apoptosis. However, this is very unlikely because our previous work has indicated that neutrophil emigration plays a much more important role than apoptosis in the removal of glomerular neutrophils in Con A glomerulonephritis (25). In addition, unlike CVF-treated rats, C6-deficient rats were not protected from neutrophil infiltration. However, despite the presence of comparable numbers of glomerular neutrophils to complement-sufficient rats with disease, they had a 25-fold reduction in the prevalence of apoptotic cells. Last, EM of apoptotic cells did not demonstrate the numerous cytoplasmic granules, which are characteristic of neutrophils (63).

The second major finding is that glomerular endothelial cell apoptosis is mediated by the C5b-9 membrane attack complex. PVG C6-deficient rats have been used to study and document the role of the C5b-9 membrane attack complex in a variety of models of renal disease (4, 11, 47). Our data indicate that the inability of the PVG C6-deficient rat to generate the C5b-9 membrane attack complex results in very marked amelioration of the rat’s disease, indicating that the overall “functional renal injury” is diminished as a consequence of the preservation of the glomerular endothelium. These data also suggest a potential for significantly inhibiting C5b-9-mediated apoptosis, not only by inhibition of C5b-9 generation but also by augmenting naturally occurring defense mechanisms to complement attack such as the cell surface complement regulatory proteins.

The third major finding is that infiltrating leukocytes do not play a prominent role in the endothelial cell apoptosis in the Con A model of glomerulonephritis. It is well documented that monocytes and macrophages can induce endothelial cell apoptosis in vitro and during development in vivo, but it is noteworthy that in our study very few apoptotic cells are evident (data not shown) when the macrophage influx is maximal at 24 h, even in C6-deficient rats, which developed a significantly increased glomerular macrophage influx com-
Is glomerular cell apoptosis functionally relevant to glomerular injury? There is now accumulating evidence in support of the concept that the inhibition of apoptosis may ameliorate acute and chronic inflammatory injury in models of disease (36). For example, in a murine model of ischemia-reperfusion injury the extent of renal injury is significantly reduced by the administration of either survival factors (insulin-like growth factor 1) or caspase inhibitors (14). Our studies indicate that renal function is significantly improved in complement-depleted or C6-deficient rats, suggesting that preservation of an intact glomerular endothelium is functionally important. It is, however, pertinent that neutrophil depletion is also renoprotective in the Con A model, and yet C6-deficient rats were found to have improved renal function despite the presence of a comparable glomerular neutrophil infiltrate to control complement-sufficient animals. How can we explain this? The Con A model of glomerulonephritis is known to be multifactorial in etiology. For example, renal function may be protected by prior platelet depletion although this also has no effect on neutrophil infiltration, thereby indicating an important proinflammatory interaction between platelets and neutrophils in this model (28). This study extends this idea by indicating that extensive endothelial cell apoptosis with inevitable endothelial denudation and exposure of glomerular basement membrane is another crucially important factor involved in the pathogenesis of renal dysfunction in this model. However, the association between the inhibition of glomerular endothelial cell apoptosis and preservation of renal function is more readily apparent in the immune-mediated thrombotic microangiopathy model because there is no significant neutrophil infiltration at any time point after the initiation of renal injury. Therefore, the results of these studies also lend support to the concept that the inhibition of apoptosis after an injurious stimulus may well protect organ function.

It should be noted that there is a small component of complement-independent apoptosis in rats systemically depleted of complement by CVF treatment and in C6-deficient rats, which are unable to generate the C5b-9 membrane attack complex. However, our data indicate that the majority of glomerular endothelial cell death in Con A glomerulonephritis and immune-mediated thrombotic microangiopathy is dependent on the C5b-9 membrane attack complex, which is in accordance with recent findings in the Thy1.1 model of mesangio proliferative glomerulonephritis (59).

What is the fate of the apoptotic glomerular endothelial cells? Apoptotic cells within solid tissues such as the renal interstitium are cleared by local or recruited phagocytes, with an estimated clearance time of ~1–2 h. It should be noted, however, that we have studied glomerular endothelial cell apoptosis early in the course of disease in these models (1 h in Con A and 4 h in immune-mediated thrombotic microangiopathy), time points that significantly precede the main influx of mononuclear cells (24 h in Con A and 3 days in immune-mediated thrombotic microangiopathy). In addition, there is evidence that recently recruited monocytes must first mature into inflammatory macrophages before they can acquire the ability to rapidly recognize and ingest apoptotic cells (61). The EM studies confirmed the light microscopic findings, with free apoptotic cells evident within the capillary lumen (Fig. 1). We would suggest that the clearance time of glomerular endothelial cells undergoing apoptosis in these models is likely to be very short indeed because the detached endothelial cells may be rapidly removed from the kidney by the circulation before being finally cleared by the reticuloendothelial system of the liver and spleen.

It is now well established that apoptosis is the preferred mode of cell death in vivo in view of its capacity to effectively delete cells without eliciting an inflammatory response. Indeed, it is becoming increasingly recognized that apoptosis plays a prominent role in situations that have been previously believed to involve mainly necrotic cell death. For example, widespread vascular endothelial cell apoptosis occurs in the generalized Shwartzman reaction in mice (induced by systemic injection of bacterial lipopolysaccharide), which results in the rapid onset of shock, disseminated intravascular coagulation, and generalized vascular occlusion; this is a scenario that would seem predilected to inducing necrotic cell injury (35). In addition, apoptosis of tubular epithelial cells in the kidney has been demonstrated in “acute tubular necrosis” caused by a variety of insults in both animals and humans (40, 42, 52). The data from this study indicating that the pore-forming C5b-9 membrane attack complex preferentially induces apoptosis of glomerular endothelial cells and not necrotic cell lysis strongly reinforce this concept.

This study does not address the mechanism underlying the induction of apoptosis by C5b-9. A body of data supports an important role for an influx of extracellular calcium into the cell in mediating the lytic effect of complement as well as the activating effects of sublytic complement on cells (5–7, 13). Furthermore, the C5b-9-mediated increase in intracellular calcium can induce the opening of the mitochondrial permeability transition pore, with subsequent loss of the mitochondrial membrane potential, which is the earliest detectable event occurring in cells destined to undergo apoptosis, preceding all morphological changes (55, 66). The resultant release of proapoptotic factors normally safely sequestered within the mitochondria, such as cytochrome c, apoptosis-inducing factor, and procaspase 9, leads to the rapid induction of apoptosis (reviewed in Ref. 23). One can therefore hypothesize that the response of a cell to complement attack is dependent on...
the number of C5b-9 membrane attack complexes inserted into the cell membrane because this may well determine intracellular calcium levels, which have been demonstrated to relate to the mode of cell death (53). For example, low numbers of C5b-9 membrane attack complexes may simply induce cell activation, e.g., proliferation, mediator release, or production of extracellular matrix. Insertion of moderate numbers may result in calcium-mediated opening of the mitochondrial permeability transition pore in a proportion of mitochondria with induction of apoptosis. Insertion of large numbers of C5b-9 membrane attack complexes, however, may induce marked elevation of intracellular calcium levels, with widespread opening of mitochondrial permeability transition pores affecting the majority of mitochondria. The associated severe fall in cellular ATP levels and the marked production of reactive oxygen species may then induce cell necrosis (24, 39). Further work, however, is required in this area.

In conclusion, this study examined the role of the C5b-9 membrane attack complex in the induction of glomerular endothelial cell apoptosis in the Con A model of immune complex glomerulonephritis in the rat. Our results indicate that glomerular endothelial cells undergo apoptosis and detach from the glomerular basement membrane. The marked reduction in glomerular endothelial cell apoptosis in C6-deficient PVG rats indicates a crucial role for the C5b-9 membrane attack complex in the induction of this mode of cell death. An identical result was found in immune-mediated thrombotic microangiopathy, a model in which inhibition of the complement regulatory protein CD59, as predicted, augmented glomerular endothelial cell apoptosis. Last, the failure of C6 deficiency to abrogate glomerular platelet and leukocyte recruitment in Con A glomerulonephritis indicated that inflammatory cells do not play a significant role in endothelial cell apoptosis in this model. These data strongly suggest that the C5b-9 membrane attack complex may play an important role in the induction of endothelial cell apoptosis in vivo, particularly in diseases associated with antiendothelial antibodies such as systemic lupus erythematosus, scleroderma, hemolytic uremic syndrome, and the systemic vasculitides.

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