Crescentic glomerulonephritis is diminished in fibrinogen-deficient mice

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Crescentic forms of glomerulonephritis are characterized by the accumulation of fibrin and cells in Bowman’s space and are associated with a rapid loss of renal function. Accumulation of fibrin in the glomerular tufts is thought to promote macrophage infiltration and glomerular injury. To directly explore the role of fibrinogen in the development of crescentic glomerulonephritis, antiglomerular basement membrane nephritis was induced in fibrinogen-deficient and control mice. Glomeruli from control mice developed severe disease including fibrin deposits, inflammatory cell accumulation, and crescent formation (46.3 ± 7.3% of glomeruli). Fibrinogen-deficient mice developed significantly milder disease with fewer glomerular crescents (24.0 ± 4.7% of glomeruli; P < 0.03). Glomerular macrophage accumulation was diminished in fibrinogen-deficient mice (0.9 ± 0.4 macrophages/glomerular cross section) relative to control mice (3.9 ± 1.4 macrophages/glomerular cross section; P < 0.03). Finally, renal function as assessed by serum creatinine was better maintained in fibrinogen-deficient mice. These results indicate that although fibrinogen is not essential for the development of glomerular crescents, it contributes significantly to the pathogenesis of crescentic glomerulonephritis by promoting glomerular macrophage accumulation and impairing filtration.

knockout; immune-mediated response

CRESCENTIC GLOMERULONEPHRITIS (GN) is usually associated with severe, rapid-onset renal injury and a poor clinical prognosis. Immune-initiated injury within glomerular capillaries and mesangium promotes leukocyte recruitment, inflammatory glomerular injury, local fibrin deposition, increased permeability of the glomerular filtration barrier, and infiltration of inflammatory cells into Bowman’s space. The combined impact of macrophage accumulation and epithelial proliferation in Bowman’s space results in the formation of cellular crescents.

Crescentic GN has many of the features of a delayed-type hypersensitivity (DTH) response (23) including prominent glomerular fibrin deposition (17) and infiltration of macrophages (2) and CD4+ T cells (29). Studies using animal models have indicated that T cells are essential to the development of crescentic GN (13, 37) with predominantly T helper type 1 (Th1)-biased immune responses resulting in crescent formation (14, 15). This suggests that Th1-directed, cell-mediated effector mechanisms such as DTH in association with coagulation-pathway activation play a central role in the development of crescentic glomerular damage.

Accumulation of glomerular fibrin and upregulation of glomerular procoagulant activity are consistent features of proliferative and crescentic forms of GN (6, 38). Fibrin and/or fibrin degradation products (FDPs) may promote the development of crescentic GN through several mechanisms. First, the deposition of fibrin and/or fibrin-platelet microthrombi within glomerular capillaries may compromise filtration across the glomerular basement membrane (GBM), ultrafiltrate flow through Bowman’s space, and glomerular blood flow. Second, fibrin is apparently chemotactic for macrophages in Bowman’s space (11) and may provide supportive matrix for inflammatory and epithelial cell migration and proliferation. Furthermore, FDPs are chemotactic for leukocytes (10, 27, 28) and may also influence macrophage accumulation in the glomerular tuft and Bowman’s space. Studies with anticoagulants and fibrinolytic agents including heparin, warfarin, tissue-type plasminogen activator (tPA), and hirudin, have demonstrated that partial inhibition of glomerular fibrin deposition protects against the formation of crescents in experimental animals (3, 7, 40). Finally, fibrin is reported to be an important mediator of injury in cutaneous DTH responses (5) with a direct role in macrophage recruitment (12).

Perhaps the most provocative findings that support a significant role of fibrin in GN are derived from studies of renal disease in mice and rabbits with either genetic.

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or drug-induced deficits in selected hemostatic factors. Elimination of key fibrinolytic system components in mice including plasminogen activator or plasminogen significantly increased glomerular fibrin, periodic acid Shiff (PAS)-positive material, macrophages, and the propensity to form crescents in an anti-GBM-induced model of GN (18). In addition, matrix metalloproteinase-9-deficient mice were recently shown to be more susceptible to anti-GBM GN due to accumulated fibrin in the glomeruli (21). In contrast, diminishing fibrinogen levels in rabbits with a snake venom component, anecrod, were shown to protect renal function, reduce glomerular inflammatory infiltrates into Bowman’s space, and decrease crescent formation (22, 32, 34). However, because anecrod results in an incomplete and temporary reduction of circulating fibrinogen (25) and may increase the formation of circulating biologically active FDPs (8), there remains significant uncertainty regarding the importance of fibrin(ogen) in promoting crescentic GN. Recent studies of anti-GBM-induced GN in thrombin receptor (protease activator receptor-1 (PAR-1))-deficient mice suggest that PAR-1 deficiency may protect against renal inflammation and crescentic GN in a manner that is at least partially independent of fibrin deposition (7).

The availability of fibrinogen-deficient (Fib−/−) mice provides the means to directly evaluate the contribution of fibrin to immune-associated glomerular injury. Fatal perinatal hemorrhagic events occur in a fraction of these mice, but mice surviving the neonatal period generally live as long as littermate control mice (31) with no sign of spontaneous renal pathology. Furthermore, unlike animals treated with anecrod, these animals have an absolute and lifelong fibrinogen deficiency. In this report, anti-GBM-induced GN was compared in Fib−/− and control (Fib+/+) mice to directly determine whether fibrin(ogen) and/or FDPs are important determinants of crescentic GN. We report that Fib−/− mice maintained lower serum creatinine levels and developed significantly less glomerular macrophages, PAS-positive material, and crescentic lesions than control mice. However, the absence of fibrin(ogen) did not completely block the progression of GN, and despite the absence of fibrin in glomerular tufts and Bowman’s space, glomerular crescents were observed in Fib−/− mice.

MATERIALS AND METHODS

Induction of GN and tissue collection. Fib−/− mice with a C57BL/6-inbred background were generated and genotyped as previously described (31). Mice were between 8 and 12 wk of age at the initiation of experiments, and all experiments were performed with female mice. Mice were presensitized with 2 mg of sheep globulin in 200 μl of Freund’s complete adjuvant (Difco Laboratories, Detroit, MI) injected subcutaneously in each flank. Ten days after presensitization, the mice (n = 26 Fib+/− and 20 Fib−/−) were injected with sheep anti-mouse GBM (5 mg iv) prepared as previously described (36). Mice dying acutely from a shocklike syndrome (equal numbers in each group) were excluded from the study (death within 2 h of anti-GBM injection, 5 Fib+/− mice and 4 Fib−/− mice; deaths between 1 and 4 days after injection, 6 Fib+/− mice and 5 Fib−/− mice). Acute deaths from hypersensitivity reactions are a feature of presensitized mouse models of anti-GBM GN and are not associated with renal pathology, increased proteinuria, or serum creatinine levels (unpublished observations). Mice surviving these early periods were included in the study and showed no evidence of the histological features of acute tubular necrosis. Nine days after disease initiation, mice were placed in metabolic cages to collect urine over a 24-h period. After 10 days, blood was collected from each mouse via the retro-orbital plexus. Mice were anesthetized, and one kidney was collected from each mouse for frozen sectioning. The other kidney was fixed in 10% neutral buffered formalin (Sigma Chemical, St. Louis, MO), processed into paraffin, and embedded for sectioning.

Histological analysis. Paraffin-embedded sections (4 μm) were cut and routinely stained with hematoxylin and eosin, PAS, and silver trichrome (Jones) stains. Glomerular cellularity was assessed by counting cells in at least 20 random equatorial glomerular cross sections (gcs) per animal. The presence of crescents (defined as the presence of three or more cell layers in Bowman’s space) was assessed in at least 60 random glomeruli and was expressed as a percentage of all glomeruli counted per animal. PAS-stained sections were scored without knowledge of mouse genotype using a semi-quantitative scale according to the content of PAS-positive material [as previously described (18)]. Briefly, glomeruli with no PAS-positive material were scored as 0, up to one-third of the cross-sectional area of the glomerulus that stained positive scored as 1, one-third to two-thirds involvement scored as 2, and greater than two-thirds involvement scored as 3. A minimum of 20 glomeruli were scored per animal.

Immunohistochemical staining for fibrin was performed on paraffin sections with rabbit anti-mouse fibrinogen antisera and detection was achieved with a Vectastain ABC kit and diaminobenzidine (DAB) substrate (Sigma). Macrophages were detected with rat anti-mouse CD11b (M1/70, Pharmingen, San Diego, CA), anti-rat Ig conjugated to horseradish peroxidase, and DAB substrate. Macrophages were quantitated by counting the number of CD11b-positive cells per glomerulus. At least 20 glomeruli per animal were counted. Glomeruli of mice not treated with anti-GBM antibodies rarely contained CD11b-positive cells.

Assessment of renal function. Serum creatinine was assessed by the alkaline picric acid method with a creatinine kit (Sigma). Proteinuria was measured using the Bradford assay on urine samples that had been collected over 24 h (4).

Detection of circulating anti-sheep globulin antibodies. Anti-sheep globulin antibodies were measured by ELISA according to a previously published protocol (14). Briefly, 96-well plates were coated with normal sheep globulin (prepared as described in Ref. 36), washed, and blocked with BSA. Plasma samples or known concentrations of mouse anti-sheep IgG (cross reactive with sheep IgG; Pierce, Rockford, IL) were added. Bound Ig was detected with biotinylated rabbit anti-mouse Ig (Vector Laboratories, Burlingame, CA), a Vectastain ABC kit (Vector), and 2,2’-azinobis-(3-ethylbenzthiazoline sulfonic acid) substrate (Boehringer Mannheim, Indianapolis, IN), and plates were read at 405 nm. In addition, IgG subtype analysis was performed by ELISA on anti-sheep-specific Ig from plasma as previously described (19).

Statistics. Results are expressed as means ± SE except where indicated. Data were analyzed by a Mann-Whitney U-test for pairwise comparisons and ANOVA for multiple comparisons.

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RESULTS

Fibrinogen is a determinant of kidney disease and survival. To directly test the prevailing hypothesis that fibrin deposition within glomeruli is an important factor in the development of crescentic GN, cohorts of Fib+/− and Fib−/− mice were immunized with sheep globulin and subsequently challenged with sheep anti-mouse GBM antibodies. Fib−/− mice have a targeted deletion within the gene encoding the Aα chain of fibrinogen and have no circulating fibrinogen. Heterozygous (Fib+/−) mice, used as controls in this study, have a fibrinogen level that is 70% of wild-type mice (because synthesis of the Bβ chain appears to limit the rate of fibrinogen production in wild-type animals) and demonstrate no phenotypic differences to wild-type mice (31). The mice were monitored for short-term survival and killed for microscopic analysis of kidney pathology 10 days after disease induction. Significant mortality was associated with induction of kidney disease in mice of both genotypes, but Fib+/− mice experienced the highest number of fatalities. Of 15 Fib+/− mice in which disease was induced, 5 mice died before day 10 (1 mouse at day 6, 2 mice at day 7, and 2 mice at day 8), and 1 moribund mouse was killed at day 8 upon the advice of a veterinarian unaware of mouse genotype. Nine Fib+/− mice survived to day 10, although 2 were moribund. In contrast, of 11 Fib−/− mice in which disease was induced, 2 were killed due to morbidity (1 each on days 8 and 9) and the 9 remaining mice appeared healthy through day 10. Morbidity and mortality (combined) were significantly higher in Fib+/− mice (8/15, 53%) than in Fib−/− mice (2/11, 18%; P < 0.03, χ² analysis). The survival advantage observed in fibrinogen-deficient mice relative to fibrinogen-expressing animals was accompanied by significantly diminished kidney disease (discussed as follows).

Histological evaluation of kidneys taken 10 days after disease induction revealed a diffuse GN had developed in both Fib+/− and Fib−/− mice (Fig. 1). In Fib+/− mice, most glomeruli were abnormal with lesion severity ranging from mild mesangial hypercellularity to profoundly necrotic glomerular lesions and/or the presence of crescents (Figs. 1 and 2A). Necrotizing changes in glomeruli from Fib+/− mice were characterized by the presence of pyknotic nuclei and increased eosinophilic matrix staining. The capillary lumens were frequently obscured by the presence of
pale-staining eosinophilic material that stained positive for fibrin, which is consistent with extensive fibrin thrombi in the glomerular capillaries. The tubules occasionally contained large protein casts, and there were mild focal chronic inflammatory infiltrates in the interstitial tissue. Focal tubular atrophy was also evident in Fib+/− animals.

Microscopic evidence of glomerular abnormalities was also found in Fib−/− mice; however, the glomerular changes observed were relatively modest compared with the Fib+/− animals. Fib−/− mice generally did not develop the pronounced expansion of the extracellular mesangial matrix, necrotizing changes, or evidence of microthrombi that were prominent in Fib+/− mice. Glomeruli of Fib−/− mice were significantly more cellular (46.7 ± 2.0 cells/gcs) despite lower numbers of infiltrating macrophages (Fig. 2B) than glomeruli of Fib+/− mice (37.9 ± 2.1 cells/gcs; P < 0.03). Both the increased proliferation of intrinsic glomerular cells and the reduced glomerular necrosis than in Fib+/− mice appeared to account for the increased cellularity in Fib−/− mice. In addition, the proportion of total glomeruli that exhibited crescentic development in Bowman’s space was significantly less in Fib−/− mice than in Fib+/− mice (Fig. 2A). Nevertheless, crescents containing immunohistochemically identified macrophages and epithelial cells were clearly evident in Fib−/− mice. Similar protein casts were seen in the tubules in both groups of mice, and a Jones stain did not show any significant argyrophilic matrix in the glomeruli of either group. Hemorrhage was not a feature of kidneys in Fib−/− mice. Although Fib−/− mice developed GN, the severity of disease development including crescent formation, cellular necrosis, and thrombosis was clearly greater in Fib+/− mice.

Fibrogen immunohistochemistry revealed a consistent accumulation of fibrin and fibrogen-related material within the glomerular tufts and crescents of Fib+/− mice (Fig. 3). Fibrin accumulation was frequently seen in occluded capillary loops of Fib+/− mice (Fig. 3, A and B). In contrast, no fibrinogen-related material was detected in the kidneys of Fib−/− mice (Fig. 3D).

Assessment of glomerular deposition of PAS-positive material. Glomeruli were graded on a scale of 0–3 for PAS-positive material accumulation, where a score of 0 indicates no increase in glomerular deposits and 3 indicates maximal accumulation of PAS-positive material. Accumulation of PAS-positive material, which is likely to include cell debris, plasma proteins, Ig deposits, and fibrin, was significantly higher in control mice (2.28 ± 0.18 arbitrary units; n = 11) compared with Fib−/− mice (1.55 ± 0.19; n = 6; P < 0.03). This difference probably reflects the absence of fibrin deposits in Fib−/− mice and the increased cellular necrosis in Fib+/− mice. Accumulation of PAS-positive material was largely associated with mesangial cells within the glomerular tuft of both genotypes. In addition, accumulation of PAS-positive material was also seen in the capillary loops of Fib+/− mice but was less common in Fib−/− mice, which suggests that fibrin deposits contribute to intraluminal thrombosis and capillary loop obliteration in Fib+/− mice.

Detection of circulating anti-sheep globulin antibodies. To demonstrate that equivalent systemic immune responses were generated in Fib−/− and control mice, circulating antibodies to sheep/goat IgG were measured in serum samples via ELISA. Increased levels of circulating antibodies to sheep IgG were detected in both Fib+/− mice (25.6 ± 4.9 μg/ml; n = 8) and Fib−/− mice (35.2 ± 6.2 μg/ml; n = 8) after induction of GN compared with untreated mice (1.0 ± 0.2 μg/ml; n = 5). IgG isotype analysis indicated that similar ratios of anti-sheep-specific IgG subtypes were present in both genotypes of mice after GN induction (as measured in OD405 units (means ± SE): total IgG: Fib+/−, 2.86 ±
IgG3: Fib+/−, 2.62 ± 0.18; Fib−/−, 0.12 ± 0.2; IgG2a: Fib+/−, 1.04 ± 0.38; Fib−/−, 0.93 ± 0.22; IgG2b: Fib+/−, 1.82 ± 0.36; Fib−/−, 1.59 ± 0.27; IgG3: Fib+/−, 1.46 ± 0.49; Fib−/−, 1.33 ± 0.34. Given that there were no significant differences between the titers of circulating specific antibodies or the IgG isotypes in Fib+/− or Fib−/− mice, the difference in severity of GN observed in mice of each genotype does not appear to be a consequence of differences in specific immune responses.

Assessment of renal function. Consistent with microscopically evident severe renal disease, serum creatinine levels were significantly higher in Fib+/− mice (67.1 ± 8.5 μM) than in Fib−/− mice (43.0 ± 2.5 μM; P < 0.03; Fig. 4). These values were elevated above the baseline creatinine value of 23.6 ± 2.9 μM, which was not different for Fib+/− or Fib−/− mice. Both Fib+/− and Fib−/− mice developed proteinuria that was significantly higher than baseline levels. Proteinuria was more pronounced in Fib−/− mice but was not significantly different from Fib+/− mice (baseline, 1.1 ± 0.5 mg/24 h; Fib−/−, 10.9 ± 7.5 mg/24 h; Fib+/−, 5.5 ± 2.1 mg/24 h; P = 0.09, Fib−/− vs. Fib+/−, means ± SD). Decreased total urine volume in Fib+/− mice (Fib+/−, 2.6 ± 1.0 ml/24 h; Fib−/−, 3.9 ± 0.7 ml/24 h) along with histological evidence of glomerular thrombus may indicate that renal filtration was impaired and may account for lower proteinuria values over a 24-h period despite more severe disease.

DISCUSSION

These studies are the first to examine the development of crescentic GN in the complete absence of either fibrinogen or FDPs and show that fibrinogen is an important factor in the development of crescents and glomerular injury. Fib−/− mice developed milder GN relative to control mice with fewer necrotic glomeruli, diminished macrophage infiltration, and a reduction in the number of glomerular crescents. Although both groups of mice developed similar systemic immune responses to the nephritogenic antigen, it appears that the accumulation of fibrin within the glomerular tuft and Bowman’s capsule of fibrinogen-expressing mice greatly exacerbates capillary damage leading to glomerular necrosis and an increased propensity toward crescent formation. These studies indicate that although fibrinogen is an important mediator of advanced renal disease in mice, it is not strictly required for either the induction of GN or crescent formation.

Glomerular procoagulant activity is elevated in crescentic GN, and the activation of the coagulation and fibrinolytic systems have been shown to contribute to disease progression (9, 35). Upregulation of tissue-factor activity leading to thrombin-mediated platelet activation, fibrin polymer formation, and the deposition of platelet-rich thrombi would be expected to promote glomerular damage and local inflammation. Consistent with this view, it has recently been demonstrated that administration of the specific thrombin inhibitor hirudin is highly protective against severe crescentic GN in mice (7). This effect was found to be at least partly due to signaling through the thrombin receptor PAR-1. However, it is likely that thrombin promotes GN through several of its known substrates, which include three G protein-coupled protease-activated receptors (PAR-1, -3, and -4); coagulation factors XI, VIII, V, and XIII, and fibrinogen; and the modulators of coagulation and fibrinolysis, protein C and thrombin-activated fibrinolysis inhibitor. The relative importance of individual targets of thrombin-mediated proteolysis in renal disease and the mechanistic details linking these substrates to severe GN remain to be fully defined.

One mechanism by which thrombin may promote the progression of GN is local proteolytic activation of PARs on endothelial cells, which results in adhesive changes that increase leukocyte and platelet deposition in glomerular vasculature. The subsequent release of proinflammatory and procoagulant factors may drive both inflammatory-cell infiltration and basement-membrane disruption leading to advanced renal disease. Thrombin may also promote progression of GN through the conversion of fibrinogen to insoluble fibrin deposits within immunologically damaged glomerular capillaries. In this model, fibrin may promote crescentic disease by providing a supportive matrix for leukocyte adhesion and cell migration into Bowman’s space. In addition, the accumulation of fibrin-rich thrombi within glomerular capillaries may cause occlusion and contribute to local mesangial and endothelial cell ischemia and subsequent necrosis. The distinctly increased macrophage recruitment, glomerular necrosis, and acellularity observed in Fib+/− mice relative to Fib−/− animals would be consistent with these concepts. Taken together with earlier findings, the studies presented here indicate that multiple targets of thrombin-mediated proteolysis are important to the progression of GN and suggest that the conversion of fibrinogen to fibrin contributes significantly to the pathology of GN.

Fibrin is a consistent and prominent feature in humans and experimental animals with severe crescentic forms of GN. Depletion of circulating fibrinogen with
ancrod in rabbits with experimentally induced GN results in significant protection against crescent formation and loss of renal function (22, 34). Macrophage infiltration into the glomerular tuft was not affected, but migration into Bowman’s space did not occur in fibrinogen-depleted animals, and subsequent crescent formation was markedly reduced (11). However, because ancrod treatment leads to incomplete and temporary fibrinogen depletion and may increase the circulating levels of fibrinogen-cleavage products (25), it may not provide a complete picture of the contribution of fibrinogen to this disease. The usage of Fib−/− mice overcomes these potential pitfalls. In the current studies, we have definitively shown that macrophage recruitment into the glomerular tuft and Bowman’s space is markedly reduced in the complete absence of fibrin, fibrinogen, and FDPs. However, the accumulation of some glomerular macrophages in mice completely lacking fibrinogen illustrates that neither soluble fibrinogen nor the local formation of fibrin matrices is essential for glomerular inflammatory infiltrates to develop. Similarly, crescent formation was significantly reduced but not absent in Fib−/− mice, which indicates that the presence of fibrinogen contributes to but is not essential for the formation of glomerular crescents.

Consistent with the histopathology of the kidneys of these mice, Fib−/− mice had lower plasma creatinine levels, which indicates preservation of glomerular filtration and reduced disease severity compared with control mice. Proteinuria, an indicator of the disruption of the glomerular filtration barrier, showed a trend toward increased levels in Fib−/− mice although these levels were not statistically different from those in Fib+/− mice (P = 0.09). This finding is consistent with studies in ancrod-treated rabbits where fibrinogen depletion preserved glomerular filtration and allowed greater protein leakage through damaged glomerular capillaries (33).

Although these studies firmly establish that fibrinogen is important in GN in vivo, a still-unresolved question involves the relative importance of soluble fibrinogen, insoluble fibrin polymers, and FDPs in renal disease progression. Interestingly, each of these fibrinogen-derived species could influence GN in ways that are not mutually exclusive. Soluble fibrinogen has many functional properties that might promote glomerular damage and inflammation including the ability to support cell-cell adhesion through integrin (e.g., αIIbβ3, α6β3, α5β1, and αMβ2) and nonintegrin (e.g., intracellular adhesion molecule-1) receptors (16, 20, 30, 39). As a dimeric molecule, fibrinogen could support the stable adhesion of leukocytes to glomerular endothelium and/or adherent platelets by acting as a “molecular bridge” between specific receptors on opposing cells. In this regard, it is notable that fibrinogen has been reported to be important in transendothelial leukocyte migration (1). The formation of fibrin polymers within the glomerular tuft or Bowman’s space could also significantly contribute to renal disease. The local deposition of an insoluble fibrin matrix could stabilize leukocyte and platelet adhesion within damaged capillaries and result in both inflammation and vasocclusion. Fibrin matrices may stimulate or support the proliferative changes that result in crescent formation. Finally, FDPs have been reported to have chemoattractant and inflammation-modulating activities (24, 26), and these proteolytic derivatives of fibrin might influence glomerular disease. The possible participation of FDPs in GN has not been excluded based on the present studies, but clearly crescentic glomerular pathologies can occur in the complete absence of FDPs. Furthermore, the recent finding that plasminogen deficiency dramatically increases the severity of GN relative to control animals would argue that at least plasmin-generated FDPs are not crucial for the development of crescentic renal disease.

In summary, this study provides definitive evidence that fibrinogen contributes to the development of crescentic GN and supports the general hypothesis that multiple hemostatic factors including tissue factor, prothrombin, plasminogen activator, and plasminogen strongly influence the progression of renal disease. The availability of a substantial number of viable mouse lines with specific hemostatic defects (e.g., P-selectin, vWF, PARs, GPIbα, integrin subunits αIIb and β3, Gaq, and coagulation factors IX, VIII, and XI) provides an opportunity to define in greater detail the impact of selected hemostatic factors on glomerular disorders. A more detailed understanding of the role and interplay of hemostatic factors in GN might suggest new therapeutic targets and adjunct therapies that might limit or reverse glomerular disease progression.

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REFERENCES


7. Cunningham MA, Rondeau E, Chen X, Coughlin SR, Holdsworth SR, and Tipping PG. Protease-activated receptor 1


