Mast Cell-Mediated Remodeling and Fibrinolytic Activity Protect against Fatal Glomerulonephritis

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*J Immunol* 2006; 176:5607-5615; doi: 10.4049/jimmunol.176.9.5607
http://www.jimmunol.org/content/176/9/5607

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Mast cells are detrimental in several inflammatory diseases; however, their physiological roles are also increasingly recognized. Recent data suggest that mast cells may also be involved in renal diseases. We therefore used congenitally mast-cell-deficient W/W\(^v\) mice and normal +/+ littermates to assess their role in anti-glomerular basement membrane-induced glomerulonephritis. Following administration of anti-glomerular basement membrane Abs, W/W\(^v\) mice exhibited increased mortality as compared with +/+ mice owing to rapid deterioration of renal function. Reconstitution of the mast cell population in W/W\(^v\) mice restored protection. This was independent of activating FcγR, as protection was also obtained using mast cells deficient in FcγR. Comparative histological analysis of kidneys showed that deterioration of renal function was caused by the presence of thick layers of subendothelial glomerular deposits in W/W\(^v\) mice, while +/+ mice or mast cell-reconstituted W/W\(^v\) mice showed significantly less. Deposits appeared during the early phase of disease and persisted thereafter, and were accompanied by enhanced macrophage recruitment. Immunohistochemical analysis revealed increased amounts of fibrin and type I collagen in W/W\(^v\) mice, which were also unable to maintain high tissue plasminogen activator and urinary-type plasminogen activator activity in urine in the heterologous phase of disease. Our results indicate that mast cells by their ability to mediate remodeling and repair functions are protective in immune complex-mediated glomerulonephritis. The Journal of Immunology, 2006, 176: 5607–5615.
cell-deficient W/Wv mice that have two mutations in the kinase domain of c-kit, the cell surface receptor for SCF, to test mast cell involvement in anti-GBM-induced glomerulonephritis (33, 34). Our results indicate that mast cells have a protective effect in immune complex-mediated glomerulonephritis by their capacity to initiate physiologic tissue remodeling and repair functions.

Materials and Methods

Cells
Chinese hamster ovary cells transfected with murine SCF were a gift from P. Dubreuil (Institut National de la Santé et de la Recherche Médicale Unité Mixte de Recherche 599, Marseille, France). They were grown in DMEM-Glutamax medium supplemented with 10% FCS, 100 IU/ml penicillin G, and 100 μg/ml streptomycin (Invitrogen Life Technologies) at 37°C in a humidified 5% CO2 incubator. Supernatant was collected and used as a source of SCF. To obtain bone marrow-derived mast cells (BMMC), suspended bone marrow-derived cells from WBB6F1/+/+ mice were cultured in RPMI 1640-Glutamax medium supplemented with 10% FCS, sodium pyruvate, nonessential amino acids, 100 IU/ml penicillin G, and 100 μg/ml streptomycin (all Invitrogen Life Technologies) containing 10 ng/ml rIL-3 (Abcys) at 37°C in a humidified 5% CO2 incubator. After 2 wk, supernatant from SCF-transfected Chinese hamster ovary cells was added at 10% as a source of SCF. BMMC were used after 5 wk and were >96% mast cells, as assessed by toluidine blue staining.

Mouse strains and reconstitution experiments
Mast cell-deficient mice were bred by mating WB/Re-ki/W/Wv+ and C57BL/6ki/W/Wv+/+/F hybrids to obtain mast cell-deficient WBB6F1/+/+/ki/W/Wv+ (W/Wv) and wild-type +/+ littermates identified by coat color (W/Wv white, +/+ black). Original breeding pairs were obtained from S. Ravetch. All mice were bred and maintained at the mouse facilities of the Jackson Laboratory. All experiments were done in accordance with national guidelines. In reconstitution experiments, 5 × 10⁶ BMMC or BMMC deficient in FcRγI/− were infused via the tail vein into W/Wv+, and recipients were studied 9 wk later. Successful reconstitution was monitored after sacrifice in peritoneum and spleen using FACS analysis (35) and toluidine blue staining, respectively. As expected, reconstitution experiments did not restore hematocrit levels, as verified following reconstitution with FcRγI−/− BMMC, demonstrating the mast cell specificity of our reconstitution experiments.

Induction of nephrotoxic nephritis
Rabbit anti-GBM Ab (nephrotoxic serum) was provided by Tanabe (36). An accelerated model of anti-GBM Ab-induced glomerulonephritis was used (32, 37). Briefly, mice were preimmunized i.p. with normal rabbit IgG (0.5 mg/20 g body weight) and CFA 5 days before i.v. administration of nephrotoxic serum through the tail vein at a dose of 0.1 ml/20 g body weight. Pilot studies in +/+ mice revealed that this dosage induced severe glomerulonephritis with functional deterioration, but did not induce fatal damage within 14 days. Except for mortality studies, mice were sacrificed at day 5 to evaluate early changes and at day 14 to evaluate late changes. No signs of glomerulonephritis were observed when normal rabbit serum was injected into +/+ and W/Wv mice. A minimum of three mice was examined per group. Kidney samples were fixed in 10% Formalin and embedded in paraffin. Some portions of the kidney were immediately frozen in Tissue-Tek OCT (Polysciences) compound for immunohistochemical analysis.

Evaluation of proteinuria and blood urea
Urine samples (10 μl) were evaluated, as described (36). Blood urea nitrogen (BUN) and creatinine levels were determined using commercially available kits (Olympus Diagnostica) by an autoanalyzer (AU400; Olympus) at IFR02 of Bichat Medical School.

Histological and immunohistochemical analysis
Kidney sections were stained with periodic acid-Schiff’s (PAS) reagent to assess histological changes and glomerular deposits in kidney tissue by light microscopy. Images were observed with a Nikon TE2000-U microscope coupled to a DXM1200F camera using a ×1 auxiliary lens and a direct ×1 C-mount. Objective magnifications are as indicated. Deposits appear as pink stained areas that contrast with adjacent glomerular structures. Kidneys were also stained with toluidine blue to evaluate presence of mast cells. Two typical pathological features were quantified: 1) To evaluate glomerular deposits, the presence of PAS-positive material within glomeruli was scored in a minimum of 50 glomeruli per mouse as follows: 0 = no deposition of PAS material, 1 = up to one-third of the cross sectional area of the glomerulus PAS−, 2 = one-third to two-thirds involvement (38). 2) Intracapillary cellular proliferation was quantitated on a scale of 1–4. A score of 1 was equivalent to 25% of the glomeruli affected by the particular morphologic change, and 4 represented involvement of 100% of the glomeruli. Values were multiplied by the number of counted glomeruli (39).

Glomerular cellularity was assessed using FITC/480 and Mac-1 (CD11b) rat antizymo Abs (Serotec) and L3T4 rat anti-mouse CD4 T cell Ab (Southern Biotechnology Associates). Rat anti-mouse IgG biotin (Southern Biotechnology Associates) and the Vectastain ABC kit (Vector Laboratories) were used for immunoperoxidase staining. Staining with secondary Ab alone was negative. Intraglomerular cell numbers were assessed using a blinded protocol. Scoring of positive cells was determined manually in high power field (×40 objective). A minimum of 50 equatorially sectioned glomeruli was assessed per animal, and the results were expressed as cells per glomerular cross section. Kidney sections were also stained with Texas red goat anti-rabbit IgG or FITC goat anti-mouse IgG (MP Biomedical) or to evaluate, respectively, heterologous or autologous Abs bound to the GBM. A minimum of 50 equatorially sectioned glomeruli were assessed per animal. Fibrin deposition was evaluated using direct staining with FITC-labeled anti-fibrin (Nordic Immunological Laboratories). The percentage of glomeruli that stained positive was determined. Collagen deposition was evaluated using rat anti-type I collagen plus anti-rat biotin (Southern Bio- technology Associates) and was scored in a minimum of 50 glomeruli per mouse as for evaluation of deposits in PAS-stained sections.

Zymography assay
Zymography to measure tissue plasminogen activator (tPA) and urinary-type tissue plasminogen activator or urokinase (uPA) activity was performed, as described (40). Briefly, 2 μl of urine samples was migrated on 12% SDS-PAGE under nonreducing conditions. After electrophoresis, the gel was soaked in 2.5% Triton X-100 for 30 min and layered onto a fibrinagarose gel containing bovine plasminogen-rich fibrinogen (7 mg/ml), bovine thrombin (40 μU/ml), and agarose (1%). Zymograms were allowed to develop for 3–24 h at 37°C. Gel photographs were scanned and quantified using the NIH Image 1.33 software program.

Data analysis
Statistical analysis was performed using an unpaired Student’s t test. Data were shown as mean ± SD, and p < 0.05 was considered to be significant.

Results
Increased mortality in mast cell-deficient mice after induction of anti-GBM glomerulonephritis
Immune complex glomerulonephritis was induced in mast cell-deficient W/Wv+ and +/+ littermates preimmunized with normal rabbit IgG by the i.v. injection of nephrotoxic anti-GBM serum. Survival was monitored over a period of 14 days. As expected from our pilot experiments with the nephrotoxic antisera, +/+ mice showed high survival over the monitoring period (Fig. 1). By contrast, W/Wv mice were highly susceptible and exhibited an increased death rate. Two peaks of mortality were observed; the first is rapid and coincides with the early heterologous phase that is initiated immediately following binding of the injected nephrotoxic antisera to the GBM; the second is somewhat delayed and coincides with the autologous phase starting around day 5 and correlating with the production of anti-rabbit IgG due to the developing immune response to injected Abs (32). To assess whether this effect was due to the absence of mast cells as opposed to other defects as a result of the mutations of c-kit in W/Wv+ mice, experiments were conducted with W/Wv− mice that were infused with BMMC to selectively repair their mast cell deficiency. After 9 wk, anti-GBM glomerulonephritis was induced. The results in Fig. 1 show that mast cell-reconstituted W/Wv+ mice behaved like
wild-type mice, revealing high survival following injection of the nephrotoxic antiserum. To assess whether the increased mortality in W/W<sup>v</sup> mice involved deteriorated renal function, proteinuria, BUN, and blood creatinine levels were measured. In agreement with the development of severe glomerulonephritis, all mice rapidly developed proteinuria following the injection of the nephrotoxic serum. However, already during the heterologous phase a tendency to higher values was observed in mast cell-deficient W/W<sup>v</sup> mice as compared with +/+ mice or W/W<sup>v</sup> mice reconstituted with BMCC (Fig. 2A) and proteinuria was significantly increased in the autologous phase. W/W<sup>v</sup> mice also showed increased levels of BUN (Fig. 2B) and creatinine (Fig. 2C) in agreement with a strong deterioration of renal function in the autologous phase. Again mice selectively reconstituted with mast cells behaved like congenic +/+ mice.

**W/W<sup>v</sup> mice exhibit major subendothelial deposits and increased macrophage infiltration**

To gain insight in the pathophysiological parameters associated with the absence of mast cells, we performed histological analysis of kidney tissue. Fig. 3A shows PAS-stained sections at days 5 and 14 after induction of anti-GBM glomerulonephritis corresponding, respectively, to the heterologous and autologous phase of the disease. As expected, +/+ mice showed diffuse signs of glomerular damage. However, already during the early heterologous phase of the disease mast cell-deficient W/W<sup>v</sup> mice exhibited marked signs of glomerular damage with a highly expanded glomerular matrix and the presence of major subendothelial deposits that are sometimes also apparent in the mesangial area. By contrast, glomeruli from +/+ mice showed only minimal deposits (Fig. 3B). These differences persisted between the early and late phases of the disease. No significant differences in intraglomerular cellularity within glomeruli were observed in kidneys between wild-type and W/W<sup>v</sup> mice (Fig. 3B). Kidney sections from mice selectively reconstituted with mast cells were assessed for late changes. Although some deposits became detectable, they appeared less pronounced than in W/W<sup>v</sup> mice. Quantification confirmed that deposits were statistically significantly different as compared with W/W<sup>v</sup> mice, but not significantly different from deposits observed in +/+ mice (Fig. 3). No differences in intraglomerular cellularity were seen among the three groups of mice (Fig. 3B).

To further assess the consequences of the absence of mast cells, we also evaluated macrophage infiltration by immunohistochemical analysis using Mac-1 and F4/80 staining, followed by quantitative analysis. As shown in Fig. 4, numbers of macrophages were slightly, but significantly, increased in W/W<sup>v</sup> mice as compared with +/+ littermates, and a similar tendency was also observed for mast cell-reconstituted W/W<sup>v</sup> mice. Although total number of intraglomerular CD4-positive T cells was low (<1/glomerula), their number was also significantly increased in W/W<sup>v</sup> mice as compared with +/+ mice (data not shown). As an increase of mast cells has been reported in a variety of inflammatory human kidney diseases (19, 20), we examined whether this holds true also for anti-GBM-induced glomerulonephritis by examining +/+ mice for mast cell infiltration using toluidine blue staining before and after induction of anti-GBM glomerulonephritis. However, mast cells were virtually absent in mouse kidneys, with occasionally one mast cell perceptible in examined kidney sections. This picture did not significantly change after induction of anti-GBM glomerulonephritis (data not shown).

**Deposits in mast cell-deficient mice are enriched in fibrin and collagen**

The striking characteristics in anti-GBM kidneys of mast cell-deficient mice were the thick subendothelial deposits, most likely responsible for the rapid loss of glomerular filtration and evolution of the disease.

![FIGURE 1.](http://www.jimmunol.org/.../W/W<sup>v</sup> mice are highly susceptible to fatal glomerulonephritis. Mast cell-deficient W/W<sup>v</sup> mice (n = 11), normal +/+ littermates (n = 11), or W/W<sup>v</sup> mice reconstituted 9 wk earlier with BMCC (n = 11) were subjected to an accelerated model of anti-GBM-induced glomerulonephritis, as described in Materials and Methods. Survival was monitored over a period of 14 days following the injection of nephrotoxic anti-GBM serum and expressed as percentage of survival.)

![FIGURE 2.](http://www.jimmunol.org/.../Renal parameters are deteriorated in W/W<sup>v</sup> mice. A. Proteinuria was measured after induction of anti-GBM glomerulonephritis over a period of 12 days in mast cell-deficient W/W<sup>v</sup> mice, normal +/+ littermates, or W/W<sup>v</sup> mice reconstituted with BMCC. BUN levels (B) and blood creatinine levels (C) were measured after induction of anti-GBM glomerulonephritis in the three groups of mice. Data are mean ± SD of blood samples taken between days 9 and 14 of at least five mice in each group. *p < 0.05.)
to endstage renal failure. We therefore investigated whether differences in heterologous and autologous Abs bound to the GBM could account for these deposits. As depicted in Fig. 5, no differences in heterologous rabbit or autologous mouse Ig deposition were detectable between /H11001/H11001/H11001, W/Wv, or mast cell-reconstituted W/Wv mice. Another protein accumulating at the site of glomerular injury is fibrin (38, 41). As mast cells are involved in many ways in the regulation of the coagulation and fibrinolysis (42, 43) and as fibrin deposition contributes to the severity of disease (44), we examined the presence of fibrin in these deposits in the late phase of the disease. Although some fibrin could be detected in glomeruli of /H11001/H11001/H11001 or mast cell-reconstituted W/Wv mice, higher amounts of this protein were seen in glomeruli from W/Wv mice (Fig. 6A). It is noteworthy to mention that these fibrin deposits were already increased during the early phase in W/Wv as compared with /+/+ mice (data not shown). The somewhat fibrillar phenotype in W/Wv mice could also be due to the deposition of fibrogenous collagen during engaged remodeling processes following immune complex-mediated injury (45). As shown in Fig. 6B, in addition to fibrin, we also detected increased amounts of intraglomerular type I collagen deposits in W/Wv mice that were significantly less in kidneys from /+/+ or mast cell-reconstituted W/Wv mice.

W/Wv mice show decreased ability to maintain fibrinolytic activity
As the increased deposition of fibrin suggested a deficiency in repair mechanisms, we examined the activity in mast cell-deficient mice of tPA and uPA, two crucial enzymes in the activation of the plasminogen/plasmin system (46). As urine presents a convenient means to measure uPA, and following renal disease induction, tPA activity (47), zymography experiments on urine samples collected from W/Wv and /+/+ mice to measure active tPA and uPA were performed. Preliminary experiments established that tPA and uPA levels were comparable in normal W/Wv and /+/+ mice (data not shown). After injection of nephrotoxic serum, high levels of both tPA and uPA activity were still present at day 1 (Fig. 7). This activity only slightly decreased at day 4 and did not significantly differ in all groups. However, at day 8, significant differences became apparent. Although /+/+ mice maintained high levels of uPA and to a lesser extent tPA, W/Wv mice were unable to maintain high activity of these enzymes.

Mast cells do not exert their protective effect through the activation of FcγR
Mast cells express several isoforms of FcγR (48, 49). Given the absence of mast cells in the kidney, we explored the possibility as to whether activation of mast cell-expressed FcγRs by Ab deposits in tissues might be involved in the protective effect. To assess the role of activating FcγR expressed on mast cells, W/Wv mice were reconstituted with BMNC derived from FcRγ-deficient mice. After 9 wk, anti-GBM glomerulonephritis was induced. The survival rate was evaluated and compared with those previously obtained for W/Wv and W/Wv mice reconstituted with wild-type BMNC (Fig. 8A). The data show that W/Wv mice reconstituted with BMNC from FcRγ-deficient mice have a higher survival rate (75%) at day 14 than W/Wv mice (20.9%) that was comparable to W/Wv mice reconstituted with wild-type BMNC (87.8%). The
Anti-GBM-induced glomerulonephritis is characterized by a heterologous phase that follows the injection of the nephrotoxic serum and the autologous phase that develops after the host mounts its immune response to the injected Abs (32, 37). The heterologous phase is characterized by a rapid and massive (within 1 h), but transient neutrophil influx that through FcR and complement-dependent mechanisms leads to glomerular injury and proteinuria (37, 50–53). The following autologous phase is characterized by the infiltration of a second set of inflammatory cells, such as macrophages and T cells. This phase develops also, albeit more slowly, in FcR-deficient mice and may involve additional factors (51). One striking observation was that protective functions of mast cells seemed to appear already during the early phase, indicating an immediate protective action of mast cells. This could involve either FcR-dependent or FcR-independent mechanisms or both. Previous experiments performed in mice deficient for the FcRγ chain that lack both activating FcγRI and III rather have shown a deleterious role of these receptors in the development of glomerulonephritis (37, 50, 51, 54), while the absence of the inhibitory FcγRIIB was shown to aggravate the disease (37). Mast cells, in addition to FcR, are known to respond to a whole variety of stimuli, some of which could be released during the initial activation of neutrophils and other cells following the binding of immune complexes, and thus act at a certain distance. Such factors may include complement fragments such as C3a or C5a, endothelin, or other inflammatory mediators that get activated in this model of glomerulonephritis (2, 3, 32). Our data favor an FcR-independent mechanism. Indeed, W/Wv mice reconstituted with mast cells deficient in FcRγ and thus lacking activating FcγRI and FcγRIII are largely protective, although a potential role of inhibitory FcγRIIB cannot be excluded. In favor of a FcR-independent mechanism is also our data showing absence of mast cell infiltration in this kidney disease model. Indeed, analysis of toluidine blue-stained tissue sections reveals no significant numbers of mast cells either before or after induction of anti-GBM glomerulonephritis. This was surprising, as it is known that human kidneys
contain increased numbers of mast cells in several renal diseases (20, 55). It agrees, however, with previous data reporting an almost complete absence of mast cells in mouse kidney tissue (55, 56). How can one then explain the marked protective effect of mast cells in this model? One plausible explanation could be that the effect results from indirect, systemic activation of factors acting at a certain distance. Evidence that mast cells can influence disease development at a certain distance has been provided in an experimental model of allergic encephalomyelitis in the brain, where the course of disease was influenced by mast cells outside the brain (57). Similar, classical anaphylaxis is a systemic response due to the activation of mast cells and/or basophils.

An important clue concerning the protective mechanism came from histological analysis, which revealed some striking features. Initial analysis showed what appeared to be highly enlarged glomeruli in W/Wv mice due to the presence of major subendothelial deposits. These deposits were seen already in the initial phase and persisted during evolution of the disease. In contrast, glomeruli from +/+ littermates or mast cell-reconstituted W/Wv mice also showed aspects of glomerular injury, but minimal deposits. Interestingly, an increase in infiltration of inflammatory macrophages was noted in mast cell-deficient mice during the late phase. From this analysis, we concluded that the deterioration of renal function in W/Wv mice was essentially associated with the presence of large deposits that may lead to an increased inflammatory response, as evidenced by macrophage infiltration.

To understand the mechanism leading to these major deposits, we examined their nature. Staining for heterologous anti-GBM Abs and autologous anti-mouse Abs bound to the glomeruli revealed no significant differences. This demonstrated that mice had received equal amounts of heterologous Abs, and also indicated that the mast cell-deficient phenotype did not affect the capacity of the mice to mount an immune response to the injected Abs, as

FIGURE 6. Mast cell-deficient W/W v mice show increased intraglomerular deposits of fibrin and type I collagen. A, Analysis of glomerular deposition of fibrin. Cryostat kidney sections from anti-GBM glomerulonephritis induced in mast cell-deficient W/W v mice, normal +/+ littermates, and W/W v mice reconstituted with BMMC after the injection of anti-GBM Ab (all from day 14) were stained with FITC anti-mouse fibrinogen Ab and analyzed by fluorescence microscopy (left panel) (×40 objective). Evaluation of deposition of fibrin in the glomeruli is shown (right panel). B, Analysis of glomerular deposition of type I collagen. Cryostat kidney sections from anti-GBM glomerulonephritis induced in mast cell-deficient W/W v mice, normal +/+ littermates, and W/W v mice reconstituted with BMMC after the injection of anti-GBM Ab (all from day 14) were stained with rat anti-type I collagen and analyzed by light microscopy (×40 objective). Evaluation of deposition of type I collagen in the glomeruli is shown (right panel). Data are mean ± SD for a minimum of four mice in each group. *, p < 0.05, significantly different from the mean value of the corresponding control.

FIGURE 7. Mast cell-deficient W/W v mice show defect in maintaining high levels of tPA and uPA in urine following induction of anti-GBM glomerulonephritis. Urine samples were collected at days 1, 4, and 8 after induction of anti-GBM glomerulonephritis from mast cell-deficient W/W v mice, normal +/+ littermates, and W/W v mice reconstituted with BMMC after the injection of anti-GBM Ab (all from day 14) and subjected to zymography analysis of tPA and uPA activity, as described in Materials and Methods. Gel photographs were scanned and quantified using the NIH Image 1.33 software program. For the purpose of comparison, tPA and uPA activities in normal +/+ littermates were arbitrarily set to 100%. These activities were comparable in normal W/W v mice. Data are mean ± SEM of four mice (three at day 8 for W/W v mice) in each group. *, p < 0.05, significantly different from the mean value of the corresponding control.
reported (58). Further screening for proteins involved in repair and remodeling processes showed that the subendothelial deposits contained large amounts of fibrin and type I collagen. Fibrin is a key factor in immune complex glomerulonephritis and has been reported to contribute to the rapid progression of the disease (38, 41, 44, 59, 60). Fibrin is dissolved by the plasminogen/plasmin system activated by either tPA or uPA. As this system also participates in proteolysis of extracellular matrix proteins, it may importantly influence the outcome of the inflammatory processes. Recent evidence suggested that mast cells mediate fibrinolytic functions, which has become an emerging concept (43). It was shown that human mast cells produce tPA in the absence of its inhibitor, and thus could directly activate plasminogen present in fibrin clots (42). However, other data show that upon stimulation, mast cells can also produce plasminogen activator inhibitor, which neutralizes tPA and uPA (61, 62). Similar immediate hypersensitivity reactions have been shown to lead to fibrin deposition (63). Thus, the potential relationships between mast cells and the plasminogen/plasmin may be complex, and the net outcome in vivo may depend on the inflammatory process. Our data rather support a net profibrinolytic action of mast cells, notably in the later autologous phase of anti-GBM glomerulonephritis. Indeed, while profibrinolytic action of mast cells, notably in the later autologous depend on the inflammatory process. Our data rather support a net proteolysis of extracellular matrix proteins, it may importantly influence the outcome of the inflammatory processes. Recent evidence suggested that mast cells mediate fibrinolytic functions, which has become an emerging concept (43). It was shown that human mast cells produce tPA in the absence of its inhibitor, and thus could directly activate plasminogen present in fibrin clots (42). However, other data show that upon stimulation, mast cells can also produce plasminogen activator inhibitor, which neutralizes tPA and uPA (61, 62). Similar immediate hypersensitivity reactions have been shown to lead to fibrin deposition (63). Thus, the potential relationships between mast cells and the plasminogen/plasmin may be complex, and the net outcome in vivo may depend on the inflammatory process. Our data rather support a net profibrinolytic action of mast cells, notably in the later autologous phase of anti-GBM glomerulonephritis. Indeed, while the +/+ mice maintain high levels of uPA and although somewhat less tPA, the activity present in urine dramatically decreases in W/Wv mice, suggesting that they are unable to maintain prolonged activity. In the earlier phase, however, both types of mice are able to turn on the activity. As high deposits of fibrin can already be detected during the early phase of disease, additional mast cell-mediated mechanisms may be operative that prevent extensive clotting. It is well known that mast cells produce heparin well recognized for its coagulation-inhibiting activity (1, 17). Mast cell proteases could also play an important role. It was shown that mice deficient in MCP-4-chymase essentially lack thrombin activity, which is one of the key proteases that activates fibrinogen (64). Likewise, tryptase released from granular stores after activation was shown to inactivate high m.w. kininogen, an initiator of the coagulation cascade (65), and to directly activate fibrinolytic prourokinase (66). Thus, our data support a net profibrinolytic function of mast cells with a protective function against the acute inflammatory injury during glomerulonephritis development. As mast cells are virtually absent in mouse kidney, this response could result from the systemic activation of tissue remodeling and repair functions by mast cells after the massive injury subsequent to the deposition of immune complexes.

Collagen is another important protein produced during tissue repair and fibrosis (67). Both pro- and antifibrotic properties have been ascribed to mast cells. Thus, heparin, histamine, and tryptase favor fibroblast growth and augment collagen synthesis (68). Lipid mediators such as LTC4, as well as certain cytokines such as IL-4, TNF-α, and TGF-β also favor the growth of fibroblasts (69, 70). Conversely, mast cell proteases such as tryptase were also found to have potent proteolytic activity toward denatured collagen or gelatin (71). Similarly, mast cell chymase has been described to activate matrix metalloproteinases 2 and 9, which also degrade denatured collagen, either by activating their proform or by degrading their natural inhibitor protein tissue inhibitor of metalloproteinase (72, 73). Human mast cells were also shown to directly produce matrix metalloproteinase-9 (74). Thus, depending on the physiological context, mast cells may either enhance or decrease collagen. In the experimental glomerulonephritis model, enhanced collagen deposition can be detected in mast cell-deficient mice, indicating that the uncoordinated production and degradation of extracellular matrix proteins following injury may be a pathologic factor that enhances renal pathology, as observed in this model. In this context, it is interesting to note that mice deficient in matrix metalloproteinase-9 were also shown to be protected against fatal anti-GBM glomerulonephritis (60).

Taken together, our data reveal an important role of mast cells in the protection against the deleterious effects of glomerular injury by immune complexes. This protective effect involves their capacity to rapidly promote repair mechanism and tissue remodeling.


