A Pathogenetic Role for Mast Cells in Experimental Crescentic Glomerulonephritis


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Mast cells infiltrate kidneys of humans with crescentic glomerulonephritis (GN), and the degree of infiltrate correlates with outcome. However, a functional role for mast cells in the pathogenesis of GN remains speculative. GN was induced by intravenous administration of sheep anti-mouse glomerular basement membrane globulin. After 21 d, systemic immune responses and disease severity were analyzed in wild-type, mast cell–deficient (W/Wv), and bone marrow–derived mast cell–reconstituted W/Wv mice (BMMC→W/Wv). There were no significant differences in the humoral response toward the nephritogenic antigen or in memory T cell number among the three groups; however, antigen-stimulated T cell IFN-γ production was significantly elevated in BMMC→W/Wv mice. Dermal delayed-type hypersensitivity in W/Wv mice was reduced compared with wild-type and BMMC→W/Wv mice. No mast cells were detected in kidneys of W/Wv mice with GN, whereas in BMMC→W/Wv mice, the numbers of renal mast cells were similar to wild-type mice with GN. W/Wv mice were protected from the development of crescentic GN, exhibiting reduced crescent formation (10 ± 1% c.f. 36 ± 2% in wild type), glomerular influx of T cells/macrophages, and interstitial infiltrate compared with wild-type mice. In contrast, BMMC→W/Wv demonstrated a similar severity of GN as wild-type mice (35 ± 2% crescentic glomeruli), accompanied by a prominent inflammatory cell infiltrate into glomeruli and interstitial areas. Glomerular expression of intercellular adhesion molecule-1 and P-selectin were reduced in W/Wv mice but restored to wild-type levels in BMMC→W/Wv mice. These findings suggest that renal mast cells mediate crescentic GN by facilitating effector cell recruitment into glomeruli via augmentation of adhesion molecule expression.


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(tryptase, chymase, histamine, and heparin), lipid-derived mediators (leukotrienes), and chemokines. Mast cells also produce a variety of cytokines that mediate delayed-type hypersensitivity (DTH) (9) and are key components of the pathogenesis of proliferative GN, injurious (e.g., TNF), and protective (e.g., IL-4) (20–22). Experimental and human GN studies have demonstrated a strong correlation between the number of interstitial mast cells and severity of interstitial injury (19,23–25). In inflammatory renal injury induced in rats by subtotal nephrectomy, a significant infiltration of mast cells in the tubulointerstitium was accompanied by increased IL-8, SCF, and TGF-β expression (26).

Although these histologic studies suggest a temporal relationship between mast cell infiltration and the development of renal injury, in vivo studies are required to prove a functional relationship. Two recent studies of mast cell–deficient rodents do not support a significant pathogenic role for mast cells in GN. In pristane-induced experimental lupus nephritis, mast cell–deficient mice developed equivalent renal disease to WT mice (27). A slightly protective role for mast cells in the development of fibrosis was suggested in rats 6 wk after the induction of puromycin aminonucleoside nephritis (28). A functional role of mast cells in the pathogenesis of crescentic GN has not been established. In these studies, we assessed the development of crescentic GN in W/Wv, WT congenic littermates, and bone marrow–derived mast cell (BMMC)-engrafted W/Wv mice. We used a well-characterized model of murine crescentic GN induced by a planted foreign antigen (Ag), in which crescentic injury is a manifestation of a T helper cell 1 (Th1)-directed DTH-mediated immune response that is dependent on effector CD4+ T cells (29–32). We demonstrate a nonredundant role for mast cells in adhesion molecule expression, infiltration of glomeruli and interstitial areas with leukocytes, and crescent formation during the course of crescentic GN.

Materials and Methods
Mice
Male WBB6/F,KitW/Wv/KitWv (W/Wv) mice and their congenic WT littermates (+/+) were purchased from The Jackson Laboratory (Bar Harbor, ME). These mice were housed in a viral room facility at Monash Medical Centre, Monash University, and monitored daily. All animal procedures were approved by the Monash Medical Centre Animal Ethics Committee B.

Mast Cell Preparation and Reconstitution
BMMC Differentiation and Cell Transfer into W/Wv
The standard technique of reconstituting the mast cell population in W/Wv mice relies on in vitro differentiation of mast cells (10,33). BMMC were differentiated in culture from bone marrow cells that were harvested from 6- to 8-wk-old WT mice. Mice were killed, and the femora and tibiae were flushed with serum-free RPMI 1640 medium. Resulting cells were cultured in medium that consisted of RPMI 1640, 15% heat-inactivated FBS, 50 U/ml penicillin, 50 µg/ml streptomycin, 2 mM l-glutamine, 1 mM sodium pyruvate, 50 µM 2-mecaprotoethanol (2-ME), 25% WEHI-3 supernatant, and 12.5 ng/ml recombinant murine (rm) SCF (R&D Systems, Gymea, NSW, Australia). Nonadherent cells were transferred to fresh culture medium once per week. After 4 to 6 wk of culture, the purity of mast cells was determined by flow cytometry for c-kit” and FcεRI” expression. Fcy receptors were blocked with rat anti-mouse CD16/32 (FcγRII/I receptor) mAb mouse (BD Fc Block; BD Biosciences PharMingen, San Diego, CA), and cells subsequently were incubated with murine IgE (10 µg/ml; BD Biosciences PharMingen) for 50 min at 4°C. Bound IgE was detected by addition of rat anti-mouse IgE-FITC (R35–72; BD Biosciences PharMingen), and c-kit expression was determined using rat anti-mouse c-kit-PE (2B8; BD Biosciences PharMingen). More than 95% of cells were positive for both IgE and c-kit and were considered to be mast cells.

Reconstitution of Mast Cells in W/Wv-Deficient Mice
BMMC (5 × 106) in 300 µl of sterile saline were injected iv into the tail vein into 9- to 10-wk-old W/Wv mice; mice were housed for 8 wk before being subjected to GN disease induction. For verifying mast cell reconstitution, small intestine, skin, and kidneys were harvested and fixed in either 4% paraformaldehyde (6 h) or Carnoy’s solution (60% ethanol, 30% chloroform, 10% acetic acid; 4 h).

Pparaformaldehyde-fixed tissues were frozen in OCT and cryostat sectioned (6 µm), whereas, Carnoy’s-fixed tissues were paraffin embedded. For quantification of MMC, 5-µm sections from Carnoy’s-fixed tissues were stained with Alcian blue/safranin O (Sigma Aldrich Pty Ltd, Castle Hill, Australia) or acidified toluidine blue (Sigma Aldrich Pty Ltd) and examined under light microscopy at ×400 magnification. Acidified toluidine blue was effective in demonstrating mast cells in skin and intestine but failed to demonstrate renal mast cells. However, occasional mast cells could be demonstrated using Alcian blue/safrin O. For assessment of whether mast cells contained significant amounts of heparin, 4 µm of Carnoy’s-fixed (deparaffinized) and 6 µm of snap-frozen (air dried) sections were fixed for 15 min in ethanol/ acetic acid (3:1) and rinsed in 100% ethanol (15 min) followed by a 10-min wash in distilled water before staining for 20 min with 0.02% (wt/vol) herberine sulfate (Sigma Aldrich Pty Ltd) in water (pH 4 with 1% citric acid). Slides were rinsed briefly in distilled water (pH 4.0), air dried, mounted with glycerol, and examined immediately with a fluorescence microscope (34). Berberine sulfates–positive cells exhibited a bright yellow fluorescence. In a blinded manner, we counted the number of positive cells per high-power field (hpf).

Initiation of GN and Experimental Design
Sheep anti-mouse glomerular basement membrane (GBM) globulin was prepared as described previously (35). Nonaccelerated autologous-phase anti-GBM GN was induced in 16- to 17-wk-old W/Wv (n = 8), W/Wv→BMMC (n = 8) and +/- (n = 8) mice by intravenous injection of 7.2 mg of anti-GBM globulin in divided doses 3 h apart (total dose = 14.4 mg). Renal injury was assessed 21 d after anti-GBM Ab administration. Results are expressed as the mean ± SEM. The statistical significance of differences between the groups was determined by one-way ANOVA, followed by Newman-Keuls post hoc test (GraphPad Prism; GraphPad Software Inc., San Diego, CA).

Assessment of Systemic Immune Responses
Humoral Immune Responses to Sheep Globulin.
Total serum anti-sheep globulin Ig and anti-sheep globulin IgG1, IgG2a, IgG2b, and IgG3 isotypes were measured by ELISA on serum that was collected at the end of the experiment. Assays were performed using microtiter plates that were coated with sheep globulin at a concentration of 10 µg/ml, with 3,3′,5,5′- tetramethyl-benzidine (Sigma Aldrich Pty Ltd) added as the chromogenic substrate; the absorbance was read at 450 nm, as described previously (36).

Induction of Dermal DTH to Sheep Globulin.
All mice that developed GN were challenged 24 h (day 20) before the end of the
experiment by intradermal injection of 500 μg of sheep globulin in 50 μl of PBS into the right-hind plantar surface of the footpad. The same dose of an irrelevant Ag (horse globulin) was injected into the contralateral footpad. Swelling was measured after 24 h using a micrometer (Mitsutoyo, Kawasaki-Shi, Japan). DTH was assessed as the difference in swelling between the two footpads and is expressed as Δ mm.

**Assessment of CD4⁺ T Cell Activation in the Spleen and Renal Lymph Node.** Single-cell suspensions were prepared from isolated spleens and draining renal lymph nodes. Cells were incubated with anti-CD44 and anti-CD4 antibodies (BD Biosciences Pharmingen); flow cytometric analysis followed, accompanied by appropriate isotype controls. The percentage of activated CD4⁺ T cells was ascertained by the proportion of cells that were positive for both antibodies.

**Assessment of Splenocyte IFN-γ Expression.** Spleens were removed aseptically, and single-cell suspensions were prepared in DMEM/5% FCS; splenocytes were incubated and cultured with sheep globulin as described previously (37). IFN-γ in resulting supernatants was measured by ELISA by using rat anti-mouse IFN-γ as the capture Ab (RA-6A2; BD Biosciences Pharmingen) and biotinylated rat anti-mouse IFN-γ (XMG1.2; BD Biosciences Pharmingen) as the capture Ab described as previously (38). The enzyme-linked immunosorbt assay was used to detect and enumerate individual cellular production of IFN-γ in vitro. The assay protocol was followed as outlined in the BD Biosciences instructions. Briefly, microwell plates were coated with anti-mouse IFN-γ (51-2525KC) in sterile PBS; microwells then were blocked with blocking solution (RPMI/10% FCS/1% PS/2-ME) and washed, and splenocytes were added (4 × 10⁶ in 100 μl) with Ag (normal sheep globulin) and incubated at 37°C for 24 h. After cells were aspirated, microwells were washed twice and the biotinylated detection Ab (biotinylated anti-mouse IFN-γ; 51-1818KZ; BD Biosciences) was added; avidin–horseradish peroxidase was applied after washing, the chromogenic substrate solution was applied, and the plate was air dried and protected from light until evaluated. Spots were enumerated manually with an inverted microscope; different sizes of spots were counted and assigned a value as follows: 1 = small, 2 = medium, and 3 = large. Results are expressed as area of positive staining/10⁶ CD4⁺ splenocytes.

**Assessment of Renal Histology**

**Glomerular and Interstitial.** For assessment of glomerular crescent formation, kidney tissue was fixed in Bouin’s fixative and embedded in paraffin, and 3-μm sections were stained with Periodic Acid-Schiff reagent. Glomeruli were considered to exhibit crescent formation when two or more layers of cells were observed in Bowman’s space. A minimum of 50 glomeruli were assessed to determine the crescent score for each animal. The number of interstitial cells in Periodic Acid-Schiff–stained renal tissue sections was also assessed. Five randomly selected cortical areas, which excluded glomeruli, were counted for each animal using a 10-μm² grid placed in the eyepiece of the microscope. Each hpf represented an area of 1 mm². Data are expressed as cells/mm² and represent the mean ± SEM, and for assessment of the interstitium, four mice from each group were randomly selected.

**Leukocyte Infiltration.** For determination of the extent of inflammatory cell influx into glomeruli, kidney tissue and spleen were fixed in periodate/lysin/paraformaldehyde. Six-micrometer cryostat-cut sections were stained to demonstrate CD4⁺ T cells and macrophages with mAb GK1.5 and M1/70, respectively, using a three-layer immunoperoxidase technique (39). A minimum of 20 equatorially sectioned glomeruli were assessed per animal, and results are expressed as cells per glomerular cross-section (gcs).

**Renal Chemokine Expression**

**RNA Extraction.** Total kidney RNA was extracted with TRizol reagent (Life Technologies, Victoria, Australia) according to the manufacturer’s protocol. The final product was air-dried and dissolved in DNase/RNase free water (Life Technologies) and stored at −70°C. The concentration of RNA was determined by spectroscopy at 260 nm.

**RNase Protection Assay.** Total kidney RNA was assessed by the RiboQuant system (BD Bioscience PharMingen) as previously reported (37). Image Gauge software (version 3.46; Fuji Photo Film, Tokyo, Japan) was used to evaluate the gel image. The expression of chemokines lymphotactin (LTN); regulated upon activation, normal T cell expressed and secreted (RANTES); macrophage inflammatory protein–1α (MIP-1α); MIP-1β; MIP-2; monocyte chemoattractant protein–1 (MCP-1); T cell activation–3 (TCA-3); and IFN-inducible protein–10 (IP-10) was measured and normalized to the housekeeping gene L32.

**Functional Assessment of Renal Injury**

**Proteinuria.** Mice were housed individually in cages to collect urine before administration of anti-GBM globulin and over the final 24 h (day 20) of the experiment. Urinary protein concentrations were determined by a modified Bradford method. Before induction of GN, all groups of mice had 24-h urine protein excretion in the normal range (0.5 to 2.0 mg/24 h).

**Serum Creatinine.** Serum creatinine concentrations were measured by an enzymatic creatininase assay.

**Measurement of Urinary Nitric Oxide Levels**

Concentrations of urinary nitrite plus nitrate were determined by Griess assay, as described previously (40); results are expressed as mg/ml.

**Results**

**Repopulation of Mast Cells with BMMC in W/W⁰ Mice**

We performed mast cell reconstitution in 9- to 10-wk-old W/W⁰ recipients by intravenous injection of BMMC (determined to be >95% pure by flow cytometry). For assessment of reconstitution of mast cells in these mice, animals were killed at 11 wk after injection, and major organs (gut, skin, and spleen) and lymph nodes were examined using histochemistry and flow cytometry. Similar patterns of mast cells were observed using an Alcian blue/Safranin O protocol in the gut of both reconstituted and +/- mice, and, as expected, no mast cells were observed in the W/W⁰ mice. Flow cytometry of splenocytes revealed a similar percentage of cells that co-expressed c-kit and FcR and lymph nodes were examined using histochemistry and flow cytometry. Similar patterns of mast cells were observed in the W/W⁰ mice. Flow cytometry of splenocytes revealed a similar percentage of cells that co-expressed c-kit and FcR in BMMC→W/W⁰ (12%) and +/- (16%) mice. Thus, the mast cell population was present 11 wk posttransplantation of BMMC, similar to previously reported findings (10,33).

**Immune Responses in W/W⁰, BMMC→W/W and +/- Mice**

Humoral immunity, measured by ELISA for total mouse anti-sheep globulin Ig in serum (Figure 1), was similar in all three groups; hence, mast cells are not required for the development of humoral immunity. Serum levels of nephritogenic Ag-specific (sheep globulin) IgG1, IgG2a, IgG2b, and IgG3 were determined by isotype-specific ELISA; there were no significant differences in IgG1, IgG2a, and IgG3 isotypes measured in the +/-, W/W⁰, and BMMC→W/W⁰ mice, although IgG2a was elevated in the BMMC→W/W⁰ mice compared with +/-
mice. Cutaneous DTH to sheep globulin was significantly impaired in W/Wv mice compared with +/+ and BMMC→W/Wv mice, which demonstrated similar swelling in response to sheep globulin. There was no difference in the percentage of CD4+ renal lymph node cells that expressed CD44, a marker for memory T cells. Finally, we assessed splenocyte production of IFN-γ in response to Ag was measured by enzyme-linked immunospot; the graph represents the area of IFN-γ corrected for the % of T cells in each well as determined by FACS (*P < 0.05 c.f. +/+; ●P < 0.05 c.f. BMMC→W/Wv; ♦ P < 0.05 c.f. W/Wv).

Identification of Renal Cell Mast Cells

The application of different staining methods revealed the existence of mature/CTMC (berberine sulfate +, Safranin O-positive staining cell) and immature/MMC (stained blue with Alcian blue) in the kidneys of diseased mice. Very few mast cells were detected in the interstitium of normal C57BL/6 and WBB6/F1-KitW+/KitW− (+/+ ) littermate control kidneys (0.05 ± 0.03 berberine sulfate-positive mast cells/hpf). In diseased +/+ mice (0.33 ± 0.08 cells/hpf) and BMMC→W/Wv mice (0.31 ± 0.07 cells/hpf), a similar number of mast cells were detected. The increase in berberine sulfate-positive mast cell numbers was noted in the interstitium, mainly in cortical areas (Figure 2). Bright fluorescence was also observed on distal tubules; this is consistent with the observation of mast cell tubulitis in which mast cells exist in the tubules (25) (data not shown). Bright fluorescence was not observed in kidneys of W/Wv mice with GN.

Alcian blue/Safranin O staining of Carnoy’s-fixed sections permitted the identification of resident MMC, which appeared blue, whereas CTMC granules stain red with Safranin O (41). Both MMC and CTMC were observed in low frequency in the interstitium of +/+ and BMMC→W/Wv mice with GN using this method (Figure 2). As expected, no mast cells were detected in kidneys of W/Wv mice with GN.

Crescentic GN in W/Wv Mice

To determine whether mast cells play a role in the development of crescentic GN, we compared the development of characteristic features (accumulation of DTH effectors, crescent formation, and renal injury) of disease in mast cell–deficient mice (W/Wv) and congenic littermates (+/+). Twenty-one days after induction of GN, glomerular crescent formation (Figure 3) was prominent in +/+ mice (36 ± 2% of glomeruli affected), with a significant inflammatory cell influx into the glomeruli (CD4+ T cells 1.6 ± 0.20 cells/gcs; macrophages 2.2 ± 0.22...
levels. However, W/Wv mice did develop proteinuria (6.3 ± 0.01), and attenuated urinary nitric oxide (0.76 ± 0.01) (Figure 4). Renal injury, indicated by proteinuria (5.0 ± 0.2 mg/ml; Figure 4). An increased number of renal mast cells similar to that observed in +/+ mice with GN was also confirmed (Figure 2). A decline of renal function was observed in BMMC→W/Wv mice, indicated by proteinuria and serum creatinine levels, although urinary nitric oxide production was only partially restored and remained significantly decreased compared with +/+ mice (Figure 5).

Intrarenal Chemokine Production and Adhesion Molecule Expression in W/Wv, +/+ , and BMMC→W/Wv Mice with GN

Given the unchanged systemic immune responses in W/Wv mice, we explored the ability of mast cells to direct recruitment of other inflammatory cells by assessing renal expression of chemokines and adhesion molecules on day 21 after GN induction. Renal expression of chemokine mRNA LTN, RANTES, MIP-1α, MIP-1β, MIP-2, MCP-1, TCA-3, and IP-10 was assessed by RNase protection assay (Table 1). We found no difference in the level of mRNA expression of any of the chemokines studied in W/Wv, +/+ , or BMMC→W/Wv, although IP-10 mRNA was significantly upregulated in BMMC→W/Wv, potentially as a result of the elevated IFN-γ observed in these mice. Although these results indicate that mast cells do not participate in chemokine upregulation, it should be noted that at this time point (day 21), we may have failed to detect the role that mast cells may exert on some of the chemokines studied, as differential chemokine expression during the nephritogenic immune response in crescentic GN has been documented (42).

Expression of renal adhesion molecules was assessed by semiquantitative scoring of P-selectin and intercellular adhesion molecule (ICAM) immunofluorescent staining. Glomerular expression of P-selectin and ICAM was significantly downregulated in W/Wv mice compared with +/+ and BMMC→W/Wv. Interstitial ICAM expression in W/Wv was also reduced, compared with BMMC→W/Wv mice but not +/+ mice (Figure 6). These results highlight pathways by which mast cells influence inflammatory cell infiltration and development of injury.

Discussion

Mast cells are recognized principally for their involvement in IgE-mediated allergic reactions; however, they also participate in innate and adaptive immunity. Mast cells are tissue-specific multifunctional cells, with diverse roles ranging from defense
against a variety of potential invasive microorganisms and parasites to mediation of autoimmune disease (43,44).

It now is generally accepted that mast cells increase in the renal fibrotic interstitium irrespective of the original pathologic process that led to the fibrotic process. A prominent rise in mast cell number has been established in a variety of renal diseases,
whereas urinary nitrite levels in W/Wv and BMMC found an elevation of nitrite in the urine of We measured urinary nitric oxide levels by Griess assay and demonstrated in W/Wv mice (the dotted line indicates normal whereas significantly lower serum creatinine levels were dem-

mast cell localization in the kidney have been observed: (46), and nephrosclerosis (25). Furthermore, three patterns of cellular rejection of renal allografts (23), diabetic nephropathy including primary and secondary nephritis (16,19,45), acute nephritic

represents the nitric oxide level observed in urine of non-

mice with GN were significantly attenuated (the dotted line

 serves in normal non-nephritic /H11001

mice; (BMMC

mice). (B) All three groups of mice with GN developed proteinuria in comparison with proteinuria ob-

duced GN in systemic autoimmunity in W/Wv mice. W/Wv mast cell–deficient; BMMC

mice with GN, W/Wv mice, and BMMC

mice with GN, W/Wv mice,

P

comparable to WT mice with

immunecomplexes and proteinuria and with 50% exhibiting
diffuse proliferative GN and 12% showing focal segmental GN


| Table 1. Renal chemokine expression relative to the housekeeping gene L32 assessed by RNAse protection assaya |
|-----------------|------------------|------------------|
|                 | +/+              | W/Wv             | BMMC→W/Wv         |
| LTN             | 3.5 ± 0.5        | 4.8 ± 0.1        | 4.9 ± 0.6         |
| RANTES           | 0.6 ± 0.04       | 1.0 ± 0.03       | 0.9 ± 0.08        |
| MIP-1β           | 5.1 ± 0.6        | 4.1 ± 0.6        | 5.1 ± 0.8         |
| MIP-1α           | 2.7 ± 0.4        | 2.7 ± 0.2        | 3.4 ± 0.5         |
| MIP-2            | 2.3 ± 0.3        | 2.9 ± 0.5        | 3.4 ± 0.3         |
| IP-10            | 1.3 ± 0.1        | 1.9 ± 0.5        | 2.9 ± 0.4         |
| MCP-1            | 8.4 ± 1.0        | 8.4 ± 1.2        | 9.1 ± 0.9         |
| TCA-3            | 2.2 ± 0.3        | 2.7 ± 0.5        | 3.5 ± 0.3         |

*aResults are expressed in arbitrary units. W/Wv, wild-type, mast cell–deficient; BMMC→W/Wv, bone marrow–derived

mast cell–reconstituted W/Wv.

Figure 5. Functional indices of renal injury and urinary nitric oxide in +/+ , W/Wv, and BMMC→W/Wv mice with GN. (A) We measured urinary nitric oxide levels by Griess assay and found an elevation of nitrite in the urine of +/+ mice with GN, whereas urinary nitrite levels in W/Wv and BMMC→W/Wv mice with GN were significantly attenuated (the dotted line represents the nitric oxide level observed in urine of non-nephritic +/+ mice). (B) All three groups of mice with GN developed proteinuria in comparison with proteinuria observed in normal non-nephritic +/+ mice, represented by the dotted line. (C) Elevated serum creatinine demonstrated impaired renal function in +/+ and BMMC→W/Wv mice, whereas significantly lower serum creatinine levels were demonstrated in W/Wv mice (the dotted line indicates normal serum creatinine in non-nephritic +/+ mice; ⋆P < 0.05 c.f. +/+; ⋈P < 0.05 c.f. BMMC→W/Wv).

including primary and secondary nephritis (16,19,45), acute cellular rejection of renal allografts (23), diabetic nephropathy (46), and nephrosclerosis (25). Furthermore, three patterns of mast cell localization in the kidney have been observed: (1) Mast cells localize in the interstitium contacting fibroblasts; (2) also in the interstitium, mast cells are in close contact with lymphocytes/macrophages; and (3) mast cells infiltrate the tubules (tubulitis), usually the distal tubules (25). Mast cells also have been detected in the interstitium, glomerulus, and tubules of rats that were administered a magnesium-deficient diet (47) and in murine renal lupus lesions (48). However, recent experimental studies of rat serum sickness nephritis, rat Masugi nephritis, and rat Thy1 nephritis failed to demonstrate the presence of mast cells in the kidney (25). In these studies, a standard technique for the identification of mast cells—tolu-
idine blue staining at pH 0.5 to 5—was used. In this study, we also used this technique and failed to see any renal mast cells. However, when we used either Alcian blue/Safranin O or berberine sulfate, mast cells were revealed in the kidney, and the phenotype of the mast cells that are involved in crescentic GN were determined to be both CTMC and MMC that are present in the cortical interstitial area but not in glomeruli; in addition, tubulitis was observed. MMC were not observed in great numbers, compared with the extent of tissue infiltration by other leukocytes. This relative low mast cell frequency in diseases in which they have been shown to play an important functional role has been observed previously (49). The predominant mast cell type that we detected in the kidney was the mature CTMC identified by berberine sulfate. This observation correlates with previous human studies that showed a significantly higher density of MC TC in forms of GN associated with fibrocellular crescents (19).

There has been considerable interest in a potential role for mast cells in the fibrotic stages of renal inflammation as they produce a number of mediators that contribute to fibrosis and collagen deposition (19). However, mast cells also produce a variety of immune regulators (cytokines and growth factors), and particularly given the demonstrated role for mast cells in other organ-specific immune disease, their role in the effector phase of inflammatory renal disease should be addressed. One recent study examined the development of autoantibody-mediated GN in systemic autoimmunity in W/Wv mice. W/Wv mice developed renal disease comparable to WT mice with immune deposits and proteinuria and with 50% exhibiting diffuse proliferative GN and 12% showing focal segmental GN.
This model is related to immune complex disease and humoral immunity. It now is recognized that most forms of human crescentic GN are associated with Th1 predominant nephritogenic immunity and the accumulation of glomerular T cell effectors (31).

To elucidate the functional pathogenic role of mast cells in crescentic GN, we studied a well-characterized model of Th1-mediated experimental anti-GBM GN and compared the development of GN in mast cell–deficient, +/+ , and BMMC→W/Wv mice. Consistent with previous observations, we also found an association between mast cell numbers and interstitial inflammation. W/Wv mice were significantly protected from GN progression, with crescent formation, glomerular T cell/macrophages, and interstitial infiltrate all substantially reduced. This suggests a role for mast cells in the development of crescentic GN. When the mast cell population was re-established in W/Wv mice (BMMC→W/Wv) systemic dermal (DTH), the numbers of DTH effector cells in glomeruli and disease severity were also restored to a similar severity observed in +/+ control mice, confirming that mast cells are crucial for the full manifestation of crescentic GN.

A role for mast cells in leukocyte recruitment was first established by the landmark studies of Askenase et al. (50) in dermal DTH. They demonstrated in transfer studies that Ag-specific T cells from W/Wv mice could induce DTH in mast cell–intact recipients, but Ag-specific T cells from normal mice could not induce DTH in W/Wv mice, confirming that the role of mast cells in DTH was to facilitate effector cell recruitment. These studies involved mast cell–deficient mice, which were then reconstituted with donor mast cells. The reconstitution allowed for the study of mast cell contribution to DTH by directly comparing mast cell–deficient versus reconstituted mice.

![Figure 6](image_url)

**Figure 6.** Renal expression of intracellular adhesion molecule 1 (ICAM-1) and P-selectin in +/+ , W/Wv, and BMMC→W/Wv mice with GN. Expression was assessed semiquantitatively by immunofluorescence, scored 0 to 3+, and mean values observed are represented graphically. (A through C) The pattern of interstitial ICAM epifluorescence seen in +/+ , W/Wv, and BMMC→W/Wv mice; notice an overall reduction of interstitial ICAM expression in W/Wv mice. (D through F) The typical pattern of glomerular ICAM-1 expression in +/+ , W/Wv, and BMMC→W/Wv mice with GN. (G through I) Glomerular P-selectin expression was striking in +/+ and BMMC→W/Wv mice with GN, whereas in W/Wv, P-selectin staining in the glomerulus was noticeably reduced (⁎P < 0.05 c.f. +/+ ; ●P < 0.05 c.f. BMMC→W/Wv).
studies in GN were consistent with these results but in addition suggest a mechanism (mast cell-regulated adhesion molecule expression) for mast cell-dependent DTH effector cell recruitment. These results collectively indicate that the important subtype that must proliferate or become activated for the full development of GN is the CTMC in rodents and the MC\textsubscript{TC} in humans. The results also indicate that mast cells mediate interstitial inflammation and that activated mast cells within the kidney synthesize mediators that participate in inflammatory cell infiltration and activation. It was surprising to note that the absence of mast cells did little to affect proteinuria but did result in reduction of serum creatinine. This observation is supported, however, by studies of secondary and primary GN in humans, in which mast cell infiltration did not correlate with urinary protein excretion but did with serum concentration of creatinine at the time of renal biopsy (16). In conclusion, this study demonstrates that interstitial mast cells modify the behaviors of adjacent kidney cells that amplify mast cell signals by releasing other soluble mediators (51), i.e., adhesion molecule expression, and thus promoting the inflammatory cascade and renal damage.

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