Role of macrophages in the fibrotic phase of rat crescentic glomerulonephritis

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1Department of Nephrology, Monash Medical Centre, Clayton, Victoria, Australia; 2Department of Medicine, Monash University, Clayton, Victoria, Australia; and 3Johnson & Johnson Pharmaceutical Research & Development, L.L.C., Spring House, Pennsylvania

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Han Y, Ma FY, Tesch GH, Manthey CL, Nikolic-Paterson DJ. Role of macrophages in the fibrotic phase of rat crescentic glomerulonephritis. Am J Physiol Renal Physiol 304: F1043–F1053, 2013. First published February 13, 2013; doi:10.1152/ajprenal.00389.2012.—The ability of macrophages to cause acute inflammatory glomerular injury is well-established; however, the role of macrophages in the fibrotic phase of chronic kidney disease remains poorly understood. This study examined the role of macrophages in the fibrotic phase of established crescentic glomerulonephritis. Nephrotoxic serum nephritis (NTN) was induced in groups of eight Wistar-Kyoto rats that were given a selective c-fms kinase inhibitor, fms-I, or vehicle alone from day 14 until being killed on day 35. Rats killed on day 14 NTN had pronounced macrophage infiltration with glomerular damage, fibrocellular crescents in 50% of glomeruli, tubulointerstitial fibrosis, and heavy proteinuria, and renal dysfunction. Glomerulosclerosis was more severe by day 35 in vehicle-treated rats, as was periglomerular and interstitial fibrosis, while proteinuria and renal dysfunction continued unabated and some parameters of tubular damage worsened. During the day 14-to-35 period, glomerular and interstitial macrophage infiltration decreased with an apparent change from a proinflammatory M1 phenotype to an alternatively activated M2 phenotype. Treatment with fms-I over days 14 to 35 selectively reduced blood monocyte numbers and abrogated glomerular and interstitial macrophage infiltration. This resulted in improved renal function, significantly reduced glomerular and interstitial fibrosis, and protection against further peritubular capillary loss. However, sustained proteinuria, tubular damage, and interstitial T cell infiltration and activation were unaffected. In conclusion, this study demonstrates that macrophages contribute to renal dysfunction and tissue damage in established crescentic glomerulonephritis as it progresses from the acute inflammatory to a chronic fibrotic phase.

c-fms; chronic kidney disease; M-CSF; M1/M2; T cells

Rapidly Progressive Glomerulonephritis (RPGN) is a devastating illness that is difficult to treat, particularly when patients present with 50% glomerular crescents, tubulointerstitial damage, and renal dysfunction (17). This group of diseases is characterized by acute inflammatory lesions that subsequently develop into glomerulosclerosis and tubulointerstitial fibrosis with a rapid progression to end-stage renal failure. Macrophage infiltration is prominent in the acute inflammatory lesions in RPGN and is associated with renal dysfunction and glomerular and tubulointerstitial histologic damage (17, 33). Infiltrating macrophages produce a variety of profibrogenic factors in these acute lesions including TNF-α, IL-1, nitric oxide synthase (NOS)-2, and macrophage migration inhibitory factor (16, 20, 30, 34), indicating a classical M1-type macrophage phenotype. However, as acute inflammation is replaced by progressive fibrosis, the role of macrophages in chronic kidney disease becomes less clear. Furthermore, there is an increasing awareness of the heterogeneous nature of macrophage responses and how this is determined by their local microenvironment (2, 8).

Experimental studies of macrophage function in RPGN have focused mainly on models of nephrotoxic serum nephritis (NTN) and lupus nephritis. A variety of strategies to block macrophage infiltration or activation during the development of inflammatory lesions has proven effective in preventing/halting active crescent formation and renal dysfunction, demonstrating that macrophages cause acute renal injury in this setting (5, 9, 11, 12, 15, 22). In contrast, the role of macrophages in established crescentic disease when predominant inflammatory lesions give way to a progressive fibrotic phase is poorly understood. Thus, it is unclear whether macrophages are a suitable target during the progressive fibrotic phase of RPGN. Studies of macrophages in progressive interstitial fibrosis in the unilateral ureteric obstruction model have produced conflicting results (3, 10, 18, 25–28), and these data cannot readily be extrapolated to a model of RPGN.

The aim of the current study was to examine macrophage function during the fibrotic phase of crescentic glomerulonephritis. To achieve this, we used an inhibitor of the macrophage-specific c-fms receptor, termed fms-I (9, 24), to deplete monocyte/macrophages in the established fibrotic phase of rat crescentic NTN.

Materials and Methods

Reagents. Mouse monoclonal antibodies were as follows: CD68 (ED1), T cell receptor (R73), vimentin, endothelium (RECA-1) (Dako, Glostrup, Denmark), and α-smooth muscle actin (α-SMA; Sigma, Castle Hill, NSW, Australia). Polyclonal antibodies were goat anti-collagen IV, rabbit anti-Wilm’s tumor antigen 1 (WT-1), and rabbit anti-PDGF-BB (Santa Cruz Biotechnology, Santa Cruz, CA). 4-cyano-1H-imidazole-2-carboxylic acid [2-cyclohex-1-enyl-4-[1-(2-methanesulfonyl)-ethyl]-piperidine-4-y]-phenyl-amide (fms-I) is a selective inhibitor of the c-fms tyrosine kinase and was synthesized by Johnson & Johnson Pharmaceutical Research & Development. Fms-I shows activity against four receptor kinases in cell-based assays with the following IC50 values (μM): c-fms, 0.0031; Flt-3, 0.098; c-kit, 0.21; Trk-A, 0.50 (9, 24).

Rat NTN. Male Wistar-Kyoto rats (180–200 g) were obtained from the Animal Resource Centre (Perth, Australia). Groups of eight rats were immunized with 1 mg of sheep IgG in Freund’s complete adjuvant followed 5 days later (day 0) by tail vein injection of sheep anti-rat glomerular basement membrane (GBM) serum. Animals were...
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KIM-1, kidney injury molecule 1; MMP-12, matrix metalloproteinase-12; NOS2, nitric oxide synthase 2; TGF, transforming growth factor.

Table 1. PCR sequence

given fms-I (10 or 30 mg/kg twice a day) or vehicle alone (20% hydroxypropyl-β-cyclodextrin in H2O) by oral gavage beginning 14 days after anti-GBM serum injection and continued twice daily until rats were killed on day 35. A group of untreated rats (NoTx) was killed on day 14 to serve as a control for disease severity at the beginning of fms-I treatment. A group of eight normal rats was also analyzed. Overnight urine (22 h) was collected on days 5, 10, 21, 28, and 35. Animal experimentation was approved by the Monash Medical Centre Animal Ethics Committee and was performed in accordance with the Australian National Health and Medical Research Council guidelines for animal experimentation.

Blood was collected at the time of death. Analysis of serum and urine creatinine used the Jaffé reaction with alkaline picrate. White blood cell counts were performed on a Cell Dyn 3500 Cell Counter (Abbott Laboratories, Abbott Park, IL). Urine protein levels were quantified using a Coomassie (Bradford) Protein Assay Kit (Thermo Fisher Scientific, Rockland, IL).

Histology. Glomerular pathology including the percentage of glomeruli with crescent formation, the size of crescents, crescent type (cellular/fibrocellular/fibrous), and the presence of Bowman’s capsule rupture was examined on periodic acid Schiff-stained sections. Each full-sized glomerular cross-section (gcs) within the section (>25) was viewed under high power (×400) and scored as follows. Glomerular crescents were defined as the presence of two or more layers of cells within Bowman’s space. The circumferential size of crescents within Bowman’s space was scored as follows: 0, no crescent; the crescent occupying one quadrant (1+), two quadrants (2+), three quadrants (3+), or all 4 quadrants (4+) of Bowman’s space. All scoring was performed on blinded slides.

Immunohistochemistry and quantification. Immunoperoxidase staining for R73+ T cells and RECA-1+ endothelium was performed on cryostat sections. Immunostaining for ED1, α-SMA, WT-1, collagen IV, and vimentin was performed on formalin-fixed paraffin sections as

Fig. 1. Glomerular pathology in periodic acid Schiff (PAS)-stained sections. A: normal rat kidney. B: day 14 nephrotoxic serum nephritis (NTN) with no treatment (NoTx) exhibiting glomerular hypercellularity, fibrocellular crescent formation (*), and disruption of Bowman’s capsule (BC). C: vehicle (Veh)-treated day 35 NTN showing glomerulosclerosis, hyaline capsular deposits, and intimal proliferation (*). D: high-dose 30-mg/kg 4-cyano-1H-imidazole-2-carboxylic acid [2-cyclohex-1-enyl-4-[1-{(2-methanesulfonyl-ethyl)-piperidin-4-yl}-phenyl]-amide (fms-I)-treated day 35 NTN showing glomerulosclerosis and fibrocellular crescent formation (*). Graphs show quantification of percentage of glomeruli with crescent formation (E), circumferential size of glomerular crescents (F), and percentage of glomeruli with rupture of Bowman’s capsule (G). Data are means ± 1 SD. *P < 0.05 for specified comparison. Original magnification: ×400 for A–D.
previously described (9). For double staining, paraffin sections were first stained with the ED1 antibody using a peroxidase-based method with development of a brown color using diaminobenzidine. Sections were then microwave treated and then stained for PDGF-BB using an alkaline phosphatase-based method with development of a purple color using the BCIP/NBP substrate (Sigma). Periglomerular myofibroblasts were assessed as the percentage of glomeruli exhibiting multiple continuous layers of α-SMA+ immunostaining.

Fig. 2. Renal fibrosis in rat NTN. Immunostaining for collagen IV is shown for normal rat kidney (A and B), day 14 NTN NoTx (C and D), vehicle-treated day 35 NTN (E and F), and high-dose 30-mg/kg fms-I-treated day 35 NTN (G and H). Graphs show quantification of area of glomerular tuft collagen IV staining (I), area of interstitial collagen IV staining (J), and interstitial volume (K). Data are means ± 1 SD. ##P < 0.01 and ###P < 0.001 vs. normal. *P < 0.05 and **P < 0.01 for specified comparisons. ###P < 0.001 vs. D14 NoTx. Original magnification: ×400 for A, C, E, G; ×160 for B, D, F, H.
The number of ED1+ macrophages, R73+ T cells, and WT-1+ podocytes per gcs was counted under high power (>50 gcs per section). The area of glomerular tuft collagen IV deposition was quantified by image analysis. High-power (>400) digital images of glomerular tuft collagen IV staining were analyzed using Image-Pro software (Media Cybernetics) to determine the area of collagen IV staining as a percentage of the total area of the glomerular tuft. Interstitial collagen IV, α-SMA, and ED1 staining was quantified by image analysis using fields covering >90% of the cortex (avoiding glomeruli and vessels) using Image-Pro software. Interstitial R73+ T cells were counted in >20 high-power interstitial fields per section. Vimentin staining of tubular cross-section was assessed in 50 consecutive fields (×250) using: 0, no positive cells; 1+, few (1 or 2) positive tubular cells in the tubule; 2+, more than 2, but less than half of cells stained; 3+, more than half of cells stained in the tubule. The peritubular capillary rarefaction index of peritubular capillary sparseness (0 to 100%) was determined by counting the numbers of squares in 12×16 grids that did not contain RECA-1+ peritubular capillary staining in at least 12 consecutive medium-power (×16) fields. The minimum possible capillary rarefaction index is 0 if every square in the grid contains a RECA-1+ capillary, while the maximal score is 100 when RECA-1+ capillaries are absent from every square in the grid. All scoring was performed on blinded slides.

Real-time RT-PCR. The RiboPure RNA isolation kit (Ambion, Austin, TX) was used to extract RNA from frozen whole kidney samples. Reverse transcription and real-time PCR was performed using Taqman probes together with an 18S or GAPDH internal control as previously described (9, 24). The IL-2 primers and probes were purchased from Applied Biosystems. The other primer pairs and probes are listed in Table 1.

Statistics. Data are shown as means ± SD and results were analyzed using parametric ANOVA with post hoc analysis with Bonferroni’s posttest for multiple comparisons. Nonparametric data were analyzed by the Kruskal-Wallis ANOVA by ranks using Dunn’s posttest for multiple comparisons. All analyses were performed using the software in GraphPad Prism 5.0 (GraphPad Software, San Diego, CA).

RESULTS

Crescentic glomerulonephritis on day 14 of NTN. Rats killed on day 14 after administration of anti-GBM serum showed marked glomerular damage including hypercellularity and hyalnosis, with crescent formation in 50% of glomeruli (Fig. 1, A, B, E). Glomerular crescents were predominantly of the fibrocellular type (89.9 ± 9.1%) with a small percentage of cellular crescents (9.9 ± 9.4%), but no fibrous crescents were seen at this time. Crescents involved one-half of the circumference of the glomerulus and were associated with rupture of Bowman’s capsule that was present in 80% of glomeruli (Fig. 1, B, F, G). Glomerulosclerosis was also evident on day 14 with a significant increase in
glomerular collagen IV deposition and α-SMA+ myofibroblast accumulation (Figs. 2, A–D, I, and 3, A, B, E), and an increase in renal mRNA levels of collagen I, plasminogen activator inhibitor (PAI)-1, and transforming growth factor (TGF)-β1 (Fig. 4, A–C). Day 14 NTN also featured tubulointerstitial damage with tubular atrophy, cast formation, and interstitial leukocyte infiltration (Fig. 5, A and B). Tubulointerstitial damage was also identified by tubular dilatation, de novo tubular vimentin expression, marked upregulation of KIM-1 mRNA levels, loss of peritubular capillaries, periglomerular and interstitial α-SMA+ myofibroblast accumulation, and collagen IV deposition (Figs. 2 to 5). In addition, heavy proteinuria with podocyte loss and renal dysfunction were established on day 14 (Fig. 6). These lesions were associated with prominent glomerular and interstitial macrophage infiltration (Fig. 7, A, B, F, G). Analysis of macrophage-specific markers using real-time PCR of whole kidney RNA showed high mRNA levels of the proinflammatory M1 markers NOS2 and matrix metalloproteinase-12 (MMP-12) and relatively low mRNA levels of M2 alternative activation markers CD163 and CD206 (Fig. 8), indicating a predominant M1-type macrophage phenotype. In addition, two-color staining identified PDGF-B expression by macrophages, including macrophage giant cells, in the glomerular tuft and in crescents on day 14 NTN (Fig. 7E). A significant glomerular and interstitial T cell infiltrate was also evident, with increased mRNA levels of IL-12 and IL-17A indicating T cell activation (Fig. 9).

Fibrotic progression of NTN over days 14 to 35. No change in the frequency of glomerular crescents was seen over days 14 to 35 in vehicle-treated NTN (Fig. 1, C and E). Fibrocellular crescents accounted for 95.5 ± 4.1% of total crescents in vehicle-treated rats at day 35 of NTN (P > 0.05 vs. day 14). However, there was some fibrous progression of crescents over this time; cellular crescents were no longer detected on day 35, and 3.5 ± 4.1% of crescents were of a fibrous type at day 35. There was no change in the size of crescents or in the frequency of Bowman’s capsule rupture (Fig. 1, F and G), but there was a significant increase in collagen IV deposition within the glomerular tuft (Fig. 2, E, F, I). Tubulointerstitial damage worsened over days 14 to 35 with increased collagen IV deposition, increased interstitial volume, and an increase in tubular dilatation and vimentin expression (Figs. 2 and 5). However, capillary rarefaction and KIM-1 mRNA levels were unchanged between days 14 and 35 (Figs. 4 and 5). Heavy proteinuria and renal dysfunction continued unabated over days 14 to 35 (Fig. 6).

An interesting feature of this progressive fibrotic phase over days 14 to 35 was the >50% reduction in macrophage accumulation, in both glomerular and interstitial compartments (Fig. 7) and reduced CD68 mRNA levels (Fig. 8A). This is suggestive of “burn out” of an acute inflammatory lesion, although kidney TNF-α mRNA levels remained elevated on day 35 (Fig. 4E). PCR analysis shows a reduction in expression of M1 markers (NOS2, MMP-12) and an increase in the expression of M2 markers (CD163, CD206) relative to CD68 mRNA levels (Fig. 8). Finally, the glomerular T cell infiltrate had resolved by day 35, but interstitial T cell infiltration and activation were unaltered (Fig. 9).
Effect of fms-I treatment over days 14 to 35 of rat NTN. Low- and high-dose fms-I treatment was well-tolerated. Both doses of fms-I abrogated glomerular and interstitial macrophage by day 35 NTN (Fig. 7), in association with a substantial reduction in blood monocyte numbers. There was no difference in blood lymphocyte or neutrophil counts between vehicle- and drug-treated rats on day 35, although high-dose fms-I caused a minor reduction in overall white blood cell counts (Fig. 7). Fms-I treatment did not affect T cell infiltration or activation (Fig. 9).

Macrophage depletion using fms-I did not change the number of glomerular crescents compared with the day 14 NoTx group, although there was a minor reduction in glomerular crescent numbers in the high-dose fms-I group on day 35 compared with vehicle-treated day 35 (Fig. 1, D and E). There was a small, but significant, reduction in crescent size with fms-I treatment on day 35, although the frequency of disruption of Bowman’s capsule was unaltered (Fig. 1, F and G). Treatment with fms-I significantly reduced glomerular and interstitial collagen IV deposition and reduced interstitial volume on day 35 NTN. This was accompanied by a reduction in the percentage of glomeruli with multiple layers of periglomerular α-SMA+ myofibroblasts, although interstitial α-SMA+ myofibroblast accumulation was unchanged (Figs. 2 and 3). This re-

Fig. 5. Tubulointerstitial damage in rat NTN. PAS-stained sections of normal rat kidney (A), day 14 NTN with NoTx (B), vehicle-treated day 35 NTN (C), and high-dose 30-mg/kg fms-I-treated day 35 NTN (D). All NTN groups showed marked tubulointerstitial damage featuring tubular dilation and atrophy, cast formation, and mononuclear cell infiltration. Graphs show quantification of tubular lumen space (E), tubular vimentin staining (F), and tubulointerstitial capillary rarefaction index (G). Data are means ± 1 SD. ***P < 0.01 and ###P < 0.001 vs. normal. *P < 0.05, **P < 0.01, and ***P < 0.001 for specified comparisons. Original magnification: ×160 for A–D.
duction in glomerular and interstitial fibrosis was associated with a reduction in renal mRNA levels of collagen I, PAI-1, and TGF-β1 (Fig. 4, A–C), and protection from further interstitial capillary loss (Fig. 5G). However, fms-I-based macrophage depletion had no effect on other parameters of tubulointerstitial damage, including tubular dilation, tubular vimentin expression, and KIM-1 mRNA levels (Figs. 4 and 5). Of note, macrophage depletion improved renal function and reduced TNF-α mRNA levels on day 35, although proteinuria continued unabated (Figs. 4 and 6).

**DISCUSSION**

This study evaluated the role of macrophages in the fibrotic phase of established experimental crescentic glomerulonephritis. This disease model showed a distinct change from inflammatory lesions to chronic fibrotic damage over days 14 to 35. No further crescent formation or disruption of Bowman’s capsule occurred after day 14 in the vehicle-treated group, indicating that this is a model of established crescentic disease. Compared with the dramatic pathologic changes seen over the first 14 days of this model, pathologic change over days 14 to 35 was relatively modest, although there was a clear increase in the severity of fibrosis in the glomerular tuft, periglomerular area, and tubulointerstitium. Notably, the aggressive macrophage infiltrate in glomerular and tubulointerstitial compartments seen on day 14 reduced by over 50% during the subsequent period of established crescentic disease. Therefore, this study enables examination of macrophage function in the chronic phase of severe kidney disease and provides a contrast with the prominent proinflammatory and injurious role that macrophages play during the development of crescentic disease during the first 14 days in this model.

Treatment with fms-I induced selective depletion of macrophages in the injured kidney as shown by the lack of effect on circulating lymphocyte and neutrophil numbers and the lack of effect upon interstitial T cell infiltration or T cell activation (IL-2 and IL-17). As previously discussed (9), the mechanisms by which fms-I treatment depleted macrophages in the injured kidney may include reduced blood monocyte numbers, reduced local proliferation, and reduced survival of macrophages within the kidney. Both doses of fms-I resulted in blood monocyte depletion, suggesting that the inability to renew the macrophage infiltrate through recruitment of blood monocytes is likely to be the major mechanism of macrophage depletion in this model of rat NTN.

Macrophage depletion via fms-I treatment over days 14 to 35 improved renal function and provided partial protection against glomerular and tubulointerstitial fibrosis and peritubular capillary rarefaction, demonstrating a significant role for macrophages in the fibrotic phase of NTN despite ongoing proteinuria and the continued presence and activation of T cells. In particular, there was a clear reduction in glomerular collagen deposition and periglomerular α-SMA+ myofibroblast accumulation, with a more minor reduction seen in overall interstitial fibrosis. The results indicate that macrophages are not required to sustain the interstitial α-SMA+ myofibroblast population, but the reduction in mRNA levels of collagen I, PAI-1, and TGF-β1 argues that macrophages are required to sustain the full fibrotic response. In addition, the observation that macrophages in the glomerulus and in crescents make PDGF-B provides one possible mechanism whereby macrophages can recruit and induce proliferation of periglomerular α-SMA+ myofibroblasts. This macrophage profibrotic function in established disease is consistent with clinical findings that interstitial macrophage numbers correlate with declining renal function, reduced capillary density, and tubulointerstitial fibrosis in human chronic kidney disease (6, 7), and in a study of renal allograft rejection in the mouse in which macrophages were shown to promote peritubular capillary loss (29). Our findings are also consistent with a study in...
Fig. 7. Macrophage infiltration in rat NTN. Immunostaining for ED1+ (CD68+) macrophages in normal rat kidney showing the presence of resident glomerular and interstitial macrophages (glomerulus (g); A); day 14 NTN with NoTx showing many macrophages in the glomerular tuft (B), within a glomerular crescent (*) and in the tubulointerstitium; vehicle-treated day 35 NTN showing reduced numbers of glomerular and interstitial macrophages (C); and high-dose 30-mg/kg fms-I-treated day 35 NTN showing abrogation of the macrophage infiltrate (D). E: 2-color immunostaining of day 14 NTN with NoTx showing ED1+ macrophages (brown) and PDGF-B (purple). While many glomerular and periglomerular cells show nuclear PDGF-B staining, glomerular macrophages are also a significant source of PDGF-B production (arrows show double positive cells, including a macrophage giant cell). Quantification of ED1 immunostaining for ED1+ macrophages per gcs (F) and percentage of interstitial area with ED1 staining (G). White blood cell (WBC) counts for total WBCs (H), monocytes (I), neutrophils (J), and lymphocytes (K). Data are means ± 1 SD. *P < 0.05, **P < 0.01, and ***P < 0.001 vs. normal. *P < 0.05, **P < 0.01, and ***P < 0.001 for specified comparisons. Original magnification: ×250 for A–D.
which CC chemokine receptor 1 (CCR1) antagonism suppressed the development of renal fibrosis in the late phase of mouse lupus nephritis, although the precise contribution of macrophages in this fibrotic process is unclear as CCR1 antagonism reduced both T cell and macrophage infiltration (1).

In marked contrast to the central role of macrophages in disruption of Bowman’s capsule and glomerular crescent formation in the first 14 days of experimental NTN, macrophage depletion over days 14 to 35 had only minor effects on the number and size of glomerular crescents. Presumably, this represents the irreversible nature of fibrocellular crescents following disruption of Bowman’s capsule (4). The small reduction in crescent number seen on day 35 with high-dose fms-I treatment may reflect resolution of the small number of early cellular crescents present on day 14.

Fig. 8. Expression of M1/M2 macrophage markers in rat NTN. RNA was extracted from whole kidney samples from normal rats, day 14 NTN with NoTx, day 35 NTN with vehicle treatment, and analyzed by real-time RT-PCR. A: CD68 mRNA levels. B–E: relative expression of macrophage markers expressed as a ratio to CD68 mRNA levels for nitric oxide synthase (NOS)2 (B), matrix metalloproteinase (MMP)-12 (C), CD163 (D), and CD206 (E). Data are means ± 1 SD. #P < 0.05 and ###P < 0.001 vs. normal. **P < 0.01 and ***P < 0.001 for specified comparisons.

Fig. 9. T cell infiltration and activation in rat NTN. Immunostaining for R73+ T cells shows R73+ T cells per gcs (A) and interstitial R73+ T cells per mm² (B). Real-time RT-PCR showing mRNA levels for IL-2 (C) and IL-17A (D). Data are means ± 1 SD. ###P < 0.001 vs. normal. ***P < 0.001 for specified comparisons.
The profibrotic role for macrophages in this model of chronic kidney disease is associated with a change from a classically activated proinflammatory M1 to an alternatively activated M2 phenotype from day 14 to 35. This shift in macrophage phenotype presumably reflects a change in the microenvironment within the injured kidney. However, it is not clear whether the M2-type macrophages seen on day 35 of disease represent a different activation pattern of newly recruited monocytes, or whether it indicates that M1-type macrophages present within the kidney on day 14 undergo phenotypic change—or a combination of both mechanisms may operate. Further studies are needed to delineate this issue.

In apparent contrast to our findings, recent studies have described an M1 to M2 change in macrophage phenotype during the resolution of tubulointerstitial damage following reversal of unilateral ureteric obstruction (19). Macrophage depletion delays recovery of kidney structure and function after ischemia-reperfusion injury indicating a reparative function for the M2-type macrophages in these lesions (21, 23). However, other studies indicate that M2-type macrophages can exert a profibrotic role in kidney disease. M2-type macrophages expressing the CD163 and CD204 markers are seen in new-onset IgA nephropathy, where they are often located in areas of glomerular and interstitial fibrosis, and their numbers correlate with mesangial expansion and interstitial fibrosis (14). Dexmethasone is a potent inducer of M2-type alternative macrophage activation and steroids are commonly used in the treatment of glomerulonephritis. In a study of rat Thy-1 mesangial proliferative nephritis, dexamethasone treatment induced M2-type alternative macrophage activation which augmented the incidence of global sclerosis and upregulated a number of profibrotic factors (13).

It is clearly too simplistic to label alternatively activated M2-type macrophages as having only a reparative role in kidney disease (2). For example, in our rat day 14-to-35 NTN study, there was ongoing severe kidney damage and the profibrotic role of macrophages in this context may still be one of attempting tissue repair, but simply failing to improve the outcome. A major difference between this NTN study and other models showing a reparative role for M2-type macrophages is that they are performed in models of transient tubular injury which allows examination of tubulointerstitial repair in the absence of ongoing renal injury. Thus, while “M2-type” macrophages are present in both situations, the very different renal microenvironment in a repairing kidney vs. one with persistent inflammatory damage means that although these M2-type macrophages may share upregulated expression of a number of specific genes, these populations are likely to have very different functional properties. Furthermore, depletion of the entire macrophage population in our model of established rat NTN means that while the overall effect of the macrophage infiltrate is profibrotic, there could be macrophage subsets with distinct pro- and anti-fibrotic actions within the overall infiltrate. An insight into this complex issue comes from adoptive transfer of ex vivo programmed M2-type macrophages that can suppress kidney injury in mouse adriamycin nephropathy despite the presence of endogenous macrophage populations (31, 32).

In conclusion, this study established that macrophages contribute to renal dysfunction and tissue damage in established crescentic glomerulonephritis as it progresses from the acute inflammatory to a chronic fibrotic phase. These findings suggest that targeting macrophage recruitment and/or activation in the chronic fibrotic phase of disease may be beneficial.

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DISCLOSURES
C. Manthey is an employee of Johnson & Johnson. D. Nikolic-Paterson has acted as a consultant for Johnson & Johnson.

AUTHOR CONTRIBUTIONS

REFERENCES


