Local macrophage and myofibroblast proliferation in progressive renal injury in the rat remnant kidney

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Abstract

Background. We have recently shown that blockade of angiotensin II activity inhibits local macrophage and myofibroblast proliferation in progressive non-immune renal injury in the rat remnant kidney. However, it is not known whether this local proliferation contributes to macrophage and myofibroblast accumulation and the development of renal injury. Therefore, we examined this issue in a detailed time-course study of the rat remnant kidney.

Methods. Groups of five rats were killed 4, 8, 12 or 16 weeks after 5/6 subtotal nephrectomy (STNx) or a sham operation. Macrophage and myofibroblast proliferation was assessed by two-colour immunostaining for ED1+ macrophages or α-smooth muscle actin (α-SMA)+positive myofibroblasts with the proliferating cell nuclear antigen (PCNA) or bromodeoxyuridine.

Results. All parameters of renal function and histology remained normal in the sham-operated controls, and no macrophage or myofibroblast accumulation was evident. In contrast, prominent macrophage accumulation developed in both the glomerulus and tubulointerstitium in STNx animals, peaking at week 12. Many ED1+ macrophages showed PCNA expression, accounting for 19–34% of the total macrophage population. There was a highly significant correlation between proliferating macrophages and total macrophage accumulation in the glomerulus \((r = 0.82, P < 0.0001)\) and tubulointerstitium \((r = 0.70, P < 0.001)\). Macrophage proliferation was largely restricted to focal areas of renal damage, such as glomerular segmental lesions and severe tubulointerstitial damage. Also, the subpopulation of proliferating macrophages gave a highly significant correlation with loss of renal function, proteinuria, and glomerular and tubulointerstitial lesions. In addition, many α-SMA myofibroblasts were evident within expanded mesangial areas and the tubulointerstitium following STNx. Interestingly, active lesions contained many large α-SMA+ cells double-stained for PCNA, accounting for 24–29% of total myofibroblasts. There was a highly significant correlation between the number of proliferating myofibroblasts and total myofibroblast accumulation during the evolution of this disease, and both populations correlated with progressive renal injury.

Conclusions. This study has shown that local proliferation is an important mechanism in both macrophage and myofibroblast accumulation during the development of renal injury in the rat remnant kidney. In addition, local macrophage proliferation is postulated as a mechanism for amplifying kidney damage in non-immune renal injury.

Key words: macrophage; myofibroblast; proliferation; fibrosis; α-smooth muscle actin; renal injury

Introduction

Subtotal nephrectomy (STNx) in the rat is a well-established model of chronic progressive renal injury, characterized by systemic hypertension, proteinuria, renal dysfunction, and fibrosis [1]. The renal haemodynamic changes such as glomerular hyperfiltration and hypertension have been well described in this model [2]. However, the cellular perturbations such as the mechanisms of renal macrophage and myofibroblast accumulation, which are pivotal for the later development of glomerulosclerosis and tubulointerstitial fibrosis, are less well characterized.

Glomerular and interstitial macrophage accumulation is a feature of most forms of human and experimental glomerulonephritis [3,4]. Macrophages have been shown to mediate renal injury in experimental models of both immune and non-immune induced glomerulonephritis [4,5]. Recent studies have demonstrated that local proliferation makes a major contribution to macrophage accumulation and the mediation
of renal injury in immunologically induced models of severe renal injury, such as acute renal allograft rejection and crescentic antiligamentous basement membrane (GBM) glomerulonephritis [6–8]. We have now extended these findings to a model of non-immune renal injury. The remnant kidney is a well-characterized rat model of non-immunologically induced renal injury in which there is prominent macrophage accumulation [9,10]. In a recent study, we identified substantial local macrophage proliferation at week 12 of the rat remnant kidney model which was significantly reduced by inhibition of the angiotensin converting enzyme [11]. This finding has raised the questions of: (a) is there a temporal association between local proliferation and the evolution of macrophage infiltration in this model of non-immune renal injury, and; (b) does local macrophage proliferation correlate with progressive renal injury in this model?

A second important cellular component of progressive renal fibrosis is the myofibroblast, which is identified by expression of α-smooth muscle actin (α-SMA) [12–15]. The induction of α-SMA expression by mesangial cells and interstitial cells has been shown to precede the development of glomerulosclerosis and tubulointerstitial fibrosis respectively, in the rat remnant kidney [9,14]. We have recently identified myofibroblast proliferation at week 12 of the rat remnant kidney model [11], suggesting that local proliferation may be important in the development of fibrosis in this model, but detailed time-course studies are needed to establish the temporal association between local proliferation and myofibroblast accumulation and the development of renal fibrosis.

The aim of the present study was, therefore, to perform a detailed study of the evolution of renal injury in the rat remnant kidney model to investigate the contribution of local proliferation to the development of macrophage and myofibroblast accumulation and the progression of renal injury.

Subjects and methods

Experimental design

Forty male Sprague–Dawley rats (200–250 g) were obtained from the Animal House, Repatriation Campus (A&R Medical Centre, Heidelberg, Australia). Twenty animals underwent subtotal nephrectomy (STNx) consisting of right subcapsular nephrectomy and infarction of approximately two-thirds of the left kidney by selective ligation of two of the three extrarenal branches of the left renal artery. At the same time 20 rats underwent a sham surgical procedure (control group) consisting of laparotomy and tactile manipulation of left kidney before wound closure. All animals were housed in a temperature (22°C) controlled room with ad libitum access to commercial standard rat chow (Norco, Lismore, NSW, Australia) and water for the duration of the study. Groups of five animals in both STNx and sham groups were killed at 4, 8, 12, or 16 weeks after surgery. A further group of six rats was killed at one week post surgery and these animals were injected with 50 mg/kg bromodeoxyuridine (BrdU) 3 h prior to sacrifice in order to label cells in the S-phase of the cell cycle. In addition, one group of five normal rats were used as the normal control. The experiment was approved by the Animal Welfare and Ethics Committee of the Austin and Repatriation Medical Centre.

Blood pressure and renal function

Systolic blood pressure was monitored weekly in conscious rats using an occlusive tail-cuff plethysmograph attached to a pneumatic pulse transducer (Narco Bio-system, Houston, TX) [15]. Prior to sacrifice, rats were housed in metabolic cages for 24 h for urine collection. Total urinary protein excretion was measured using the Coomasie brilliant blue method. At the time of sacrifice, serum was collected for measurement of urea and creatinine concentrations.

Histology

Renal tissues were fixed in 10% neutral buffered formalin and embedded in paraffin. Four-micrometre paraaffin sections were stained with haematoxylin and periodic acid–Schiff (PAS), or with Masson’s trichrome. Glomerulosclerosis was assessed on PAS-stained sections, using a slightly modified version of the semiquantitative scoring method of Raij et al. [16]. The extent of tubulointerstitial injury was evaluated on trichrome-stained sections to assess the relative proportions of tubular dilatation, tubular atrophy, and interstitial fibrosis, using a standard point-counting technique [17]. Data were expressed as percentage of tubules exhibiting tubular lesions (tubular dilatation, atrophy, or fibrosis).

Antibodies

Monoclonal antibodies (mAb) used in this study were: ED1, anti-rat CD68 which recognizes most monocytes/macrophages [18,19]; 1A4, anti-smooth-muscle α-actin isoform [20]; PC10, antiproliferating cell nuclear antigen (PCNA) which recognizes cells in G1, S, and G2 phases [21] and M744, anti-bromodeoxyuridine (Dako Ltd, Glostrup, Denmark). In addition, peroxidase-conjugated goat anti-mouse immunoglobulin, alkaline phosphatase-conjugated goat anti-mouse immunoglobulin, mouse peroxidase–anti-peroxidase complexes (PAP) and mouse alkaline phosphatase–anti-alkaline phosphatase complexes (APAAP) were used. All antibodies were purchased from Dakopatts (Glostrup, Denmark).

Immunohistochemistry

Two-colour immunohistochemistry staining was performed using a microwave-based technique [22]. Briefly, paraffin sections (4 μm) of formalin-fixed tissues were treated by two rounds of microwave oven heating in 0.01 M sodium citrate pH 6.0, each lasting for 5 min, at 2450 MHz and 800 Watts power. This treatment was used to retrieve CD68, PCNA and BrdU antigens. Following microwave treatment, slides were washed twice with PBS and preincubated with 10% fetal calf serum (FCS) and 10% normal goat serum in PBS for 20 min, drained, and incubated with the ED1 or 1A4 mAb overnight at 4°C. Endogenous peroxidase was inactivated by incubation in 0.3% H2O2 in methanol, and sections were incubated with peroxidase-conjugated goat anti-mouse IgG followed by mouse PAP, and developed with 3,3-diaminobenzidine to produce a brown colour. At this point, slides were treated by another two rounds of micro-
wave heating as described above. This treatment was used to denature IgG molecules bound to tissue sections to prevent antibody cross-reactivity and improve mAb access to the nuclei. Following a second preincubation as above, sections were incubated with the PC-10 (1:1000) or M744 (1:100) mAb overnight at 4°C, washed in PBS, and then incubated sequentially with goat anti-mouse IgG and mouse APAAP and developed with Fast blue BB Base (Sigma Chemical Co, St Louis, MO, USA). Sections were counterstained with periodic acid–Schiff reagent without haematoxylin, and coverslipped in an aqueous mounting medium. The same procedure was used for double-staining of z-SMA and PCNA, except that no microwave treatment was employed before labelling with the 1A4 mAb.

Quantitation of immunohistochemistry

Labelled cells in tissue sections were scored as previously described [8]. Briefly, the number of total macrophages (ED1+ cells), proliferating macrophages (ED1+PCNA+ cells), total myofibroblasts (z-SMA+ cells), and proliferating myofibroblasts (z-SMA+PCNA+ cells) were counted under high power (×40) in at least 20 glomerular cross-sections per animal. In the cortical interstitium, the number of labelled cells were counted in at least 20 consecutive high power fields (×40) by means of a 0.02 mm2 graticule fitted in the eyepiece of microscope moving from outer cortex to inner cortex. Data are presented as the mean ± SEM per glomerular cross-section or per mm2.

All morphological analysis and cell counting was performed on blinded slides and the areas evaluated were in the renal cortex at a site distant from the surgical infarct.

Statistical analysis

The number of glomerular and interstitial macrophages and myofibroblasts in the STNx and sham groups were analysed by one-way analysis of variance (ANOVA) using the Complete Statistical Analysis program (CSS, Statsoft, Tulsa, OK, USA). The number of macrophages and myofibroblasts were correlated with blood pressure, histological damage, and renal function using either the single parametric Pearson correlation coefficient or the non-parametric Spearman’s rank coefficient from CSS.

Results

Histology, renal function and blood pressure

The STNx rats had an elevated systolic blood pressure, and developed chronic renal failure with a significant rise in serum urea and creatinine, heavy proteinuria, and progressive glomerulosclerosis and tubulointerstitial injury (Table 1). All of these parameters remained within the normal range in the sham-operated control group for the duration of the experiment.

Local macrophage accumulation and proliferation

A small resident population of ED1+ macrophages was present in glomeruli and the tubulointerstitium in normal rat kidney and in sham-operated controls. However, only very rare ED1+PCNA+ proliferating macrophages were seen in normal rats or sham-operated controls (Figures 1a and 2). In contrast, there was a marked accumulation of ED1+ macrophages in both glomeruli and the tubulointerstitium over the 16 week time course of the disease model. Double staining demonstrated that many ED1+ macrophages in both compartments were proliferating on the basis of PCNA expression (Figure 1b,c), accounting for 19–34% of the total macrophage population over weeks 4 to 16 (Figure 2). Macrophage proliferation was largely restricted to areas of focal renal damage, such as glomerular focal and segmental lesions (including hypercellularity), areas of Bowman’s capsule rupture and areas of severe tubulointerstitial damage with focal tubular dilatation, atrophy and interstitial fibrosis (Figure 1c). This became more prominent as the severity of renal damage increased during the evolution of the disease. To examine earlier events in this disease model, a group of animals were killed at week 1 post STNx. Local macrophage proliferation, identified by incorporation of BrdU into ED1+ macrophages (Figure 1d), was evident at even this early time point. The temporal association between local proliferation and macrophage accumulation in this model was shown by the highly significant correlation between the number of proliferating macrophages (ED1+PCNA+ cells) over the 16 week period and total macrophage accumulation in both glomeruli (r = 0.82, P < 0.0001) and the tubulointerstitium (r = 0.70, P < 0.001).

Correlation of macrophage accumulation and proliferation with renal functional and histological injury

Immunohistochemistry studies showed a temporal and spatial association between macrophage proliferation and accumulation with the evolution of renal damage in this disease model. In addition, we performed a correlation analysis between the numbers of proliferating and total macrophages with different parameters of clinical and histological injury over the 16 week time-course of the disease model. Glomerular ED1+ macrophage accumulation correlated significantly with the development of severe proteinuria over the 16-week period, but failed to correlate with other disease parameters (Table 2). In contrast, the subpopulation of glomerular ED1+PCNA+ proliferating macrophages correlated with elevated blood pressure, chronic renal failure, proteinuria and glomerular lesions. A similar picture was seen within the tubulointerstitium where the subpopulation of proliferating macrophages gave a much better correlation with parameters of renal function and histology compared to total macrophage accumulation (Table 2).

z-SMA expression and myofibroblast proliferation

No z-SMA was detected in glomeruli or the tubulointerstitium of kidneys from normal or sham-operated rats, except for the strong staining of z-SMA in vascular smooth muscle cells (Figure 1e). In contrast, there was strong staining of z-SMA within expanded
Table 1. Blood pressure, renal function, and histology in the rat remnant kidney

<table>
<thead>
<tr>
<th>Weeks</th>
<th>Blood pressure/renal function</th>
<th>Histology</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Final BP (mmHg)</td>
<td>Final serum urea (mmol/l)</td>
</tr>
<tr>
<td>STNx</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>202.8 ± 9.2b</td>
<td>24.9 ± 8.7b</td>
</tr>
<tr>
<td>8</td>
<td>173.6 ± 14.2b</td>
<td>15.3 ± 14.4b</td>
</tr>
<tr>
<td>12</td>
<td>206.8 ± 18.3b</td>
<td>22.9 ± 8.7b</td>
</tr>
<tr>
<td>16</td>
<td>188.4 ± 24.2b</td>
<td>18.3 ± 4.2b</td>
</tr>
<tr>
<td>Sham</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>137.2 ± 8.9</td>
<td>7.6 ± 0.4</td>
</tr>
<tr>
<td>8</td>
<td>138.8 ± 18.3</td>
<td>6.7 ± 0.5</td>
</tr>
<tr>
<td>12</td>
<td>136.8 ± 7.5</td>
<td>7.1 ± 0.4</td>
</tr>
<tr>
<td>16</td>
<td>160.4 ± 5.6</td>
<td>6.8 ± 1.1</td>
</tr>
<tr>
<td>Normal</td>
<td>125 ± 4.2</td>
<td>6.9 ± 0.5</td>
</tr>
</tbody>
</table>

Data are expressed as mean ± SD for groups of five animals. GSL, glomerular segmental lesions. Time-matched STNx and sham-operated control groups were compared by one-way analysis of variance (ANOVA): *P < 0.05; **P < 0.01.

Correlation of α-SMA expression and myofibroblast proliferation with renal functional and histological injury

The relationship between the appearance of proliferating and total α-SMA+ myofibroblast populations with parameters of clinical and histological renal injury was examined over the entire disease course. There was a significant correlation between glomerular α-SMA+ cells and loss of renal function, proteinuria and glomerular lesions (Table 3). Tubulointerstitial myofibroblasts also correlated significantly with disease parameters, particularly increased serum creatinine, proteinuria and tubular lesions. In both compartments, the subpopulation of proliferating α-SMA+ PCNA+ cells gave a significant correlation with disease parameters, comparable to that seen with the total α-SMA+ myofibroblast population (Table 3).

Discussion

This study has identified substantial local proliferation of both macrophages and myofibroblasts during the evolution of glomerular and tubulointerstitial injury following subtotal nephrectomy in the rat. The relevance of these findings to progressive renal injury in this and other disease models is considered below.
Fig. 2a,b. Quantitation the macrophage accumulation and proliferation following STNx in the rat. The number of ED1\(^+\) macrophages (squares) and ED1\(^+\) PCNA\(^+\) proliferating macrophages (circles) were scored in animals undergoing STNx (closed symbols) or sham-surgery (open symbols) in (a) the glomerulus, and (b) the cortical tubulointerstitium. Data are expressed as mean ± SEM. *P<0.05; **P<0.01 vs Sham; \(a\)P<0.05; \(b\)P<0.01 vs normal, by ANOVA.

Table 2. Correction analysis of macrophage accumulation, macrophage proliferation, and disease parameters

<table>
<thead>
<tr>
<th></th>
<th>Glomerulus</th>
<th>Tubulointerstitium</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ED1(^+)</td>
<td>ED1(^+) PCNA(^+)</td>
</tr>
<tr>
<td>BP/renal function</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Final blood pressure</td>
<td>0.36</td>
<td>0.45(a)</td>
</tr>
<tr>
<td>Final serum urea</td>
<td>0.42</td>
<td>0.52(a)</td>
</tr>
<tr>
<td>Final serum creatinine</td>
<td>0.42</td>
<td>0.69(b)</td>
</tr>
<tr>
<td>Proteinuria</td>
<td>0.45(a)</td>
<td>0.62(b)</td>
</tr>
<tr>
<td>Histology</td>
<td></td>
<td></td>
</tr>
<tr>
<td>GSL score</td>
<td>0.34</td>
<td>0.52(a)</td>
</tr>
<tr>
<td>Tubular lesions</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>

Cell counts from 25 animals in the study were compared with measurements of systolic blood pressure (BP), serum urea and creatinine, and proteinuria by the parametric single Pearson coefficient, and compared with semi-quantitative scoring of renal histology (GSL, glomerular segmental lesions, and tubular lesions) by the non-parametric Spearman’s Rank correlation coefficient. The correlation coefficients (\(r\) values) are shown: *P<0.05; \(a\)P<0.05.

Fig. 3a,b. Quantitation the myofibroblast accumulation and proliferation following STNx in the rat. The number of \(\alpha\)-SMA\(^+\) myofibroblasts (squares) and \(\alpha\)-SMA\(^+\) PCNA\(^+\) proliferating myofibroblasts (circles) were scored in animals undergoing STNx (closed symbols) or sham surgery (open symbols) in (a) the glomerulus, and (b) the cortical tubulointerstitium. Data are expressed as mean ± SEM. *P<0.05; **P<0.01 vs Sham; \(a\)P<0.05; \(b\)P<0.01 vs normal, by ANOVA.

Table 3. Correlation between myofibroblast subpopulation and blood pressure/renal function or histology in STNx group

<table>
<thead>
<tr>
<th></th>
<th>Glomerulus</th>
<th>Tubulointerstitium</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(\alpha)-SMA(^+)</td>
<td>(\alpha)-SMA(^+) PCNA(^+)</td>
</tr>
<tr>
<td>BP/renal function</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Final blood pressure</td>
<td>0.26</td>
<td>0.26</td>
</tr>
<tr>
<td>Final serum urea</td>
<td>0.60(b)</td>
<td>0.62(b)</td>
</tr>
<tr>
<td>Final serum creatinine</td>
<td>0.66(b)</td>
<td>0.64(b)</td>
</tr>
<tr>
<td>Proteinuria</td>
<td>0.47(a)</td>
<td>0.42</td>
</tr>
<tr>
<td>Histology</td>
<td></td>
<td></td>
</tr>
<tr>
<td>GSL score</td>
<td>0.46(a)</td>
<td>0.56(b)</td>
</tr>
<tr>
<td>Tubular lesions</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>

Cell counts from 25 animals in the study were compared with measurements of systolic blood pressure (BP), serum urea and creatinine and proteinuria by the parametric single Pearson coefficient and compared with semi-quantitative scoring of renal histology (glomerular segmental lesions and tubular lesions) by the non-parametric Spearman’s Rank correlation coefficient. The correlation coefficients (\(r\) values) are shown: *P<0.05; \(a\)P<0.05.
The accumulation of monocyte/macrophages plays an important role both in mediating renal injury and in regulating the fibrotic response to renal injury [4,23]. A number of recent studies have shown that local proliferation of inflammatory macrophages plays an important role in macrophage accumulation and renal injury in immunologically-induced models of severe renal injury, such as acute alloantigen rejection and crescentic glomerulonephritis [6–8]. The degree of local macrophage proliferation is related to the severity of renal injury as demonstrated by the low levels of proliferation seen in immune-complex glomerulonephritis and acute mesangioproliferative nephritis [24–26]. The current study has shown that local proliferation is an important mechanism of macrophage accumulation during the evolution of severe non-immune renal injury in the rat remnant kidney. As in severe immunological renal injury, the rat remnant kidney model showed a tight association of local proliferation with areas of tissue damage and a highly significant correlation between local macrophage proliferation and progressive renal injury, supporting the postulate that local proliferation is a mechanism for amplifying macrophage accumulation and macrophage-mediated renal injury, irrespective of the nature of the initial renal insult. Indeed, the percentage of proliferating macrophages increased in the more severely injured kidneys, which was reflected by the better correlation of proliferating macrophage with renal injury than that of the total macrophage population. One point to note is an apparent discrepancy between the current study and that of Klem et al. [9], in which few macrophages or z-SMA+ myofibroblasts were found to express PCNA during a 10-week course following 5/6th nephrectomy in rats. This difference may be related to the severity of the renal injury induced by renal ablation and/or in the sensitivity of the antigen-retrieval technique used for immunostaining of the PCNA antigen, or the specific anti-PCNA antibody used. The use of BrdU as a second marker of cell proliferation confirmed the presence of significant local macrophage proliferation as early as week 1 in the current study.

Although the mechanism driving local macrophage proliferation in renal disease remains to be elucidated, a role for macrophage colony-stimulating factor (M-CSF) has been suggested in studies of murine lupus nephritis and rat crescentic glomerulonephritis [27–29]. The question of whether M-CSF participates in local macrophage proliferation in non-immune renal injury is currently under investigation in our laboratory.

Myofibroblasts, defined phenotypically by their expression of z-SMA, play a key role in progressive glomerulosclerosis and interstitial fibrosis in renal disease [30]. A number of studies have shown that glomerular mesangial cells exhibit de novo z-SMA expression as they transdifferentiate into myofibroblasts in association with the development of glomerulosclerosis, while the appearance of many z-SMA+ myofibroblasts is a characteristic of interstitial fibrosis in both human and experimental glomerulonephritis [9,12,31–35]. We found a progressive increase in glomerular and interstitial z-SMA+ myofibroblasts following STNx, consistent with previous studies in this model in which the appearance of z-SMA+ myofibroblasts was shown to precede the development of glomerulosclerosis and tubulointerstitial fibrosis [9,34]. While glomerular mesangial cells are thought to give rise to the z-SMA+ cells seen in the glomerulus, little is known of how z-SMA+ myofibroblasts accumulate within the tubulointerstitium. The temporal association between increasing levels of proliferation and the number of tubulointerstitial of z-SMA+ myofibroblasts suggests that local proliferation is an important mechanism driving myofibroblast accumulation in the rat remnant kidney. This marked proliferation of z-SMA+ myofibroblasts indicates that these are not terminally differentiated cells. This is consistent with a study of E. coli-induced renal scarring in the rat, in which high levels of interstitial myofibroblast proliferation were demonstrated using bromodeoxyuridine incorporation as a marker of cell division [35]. A second important finding was the significant correlation between myofibroblast proliferation and accumulation with renal impairment, glomerular injury and histologic damage. While the appearance of myofibroblasts is usually considered to be a repair response following tissue injury that promotes renal injury through matrix deposition and progressive ischaemia, it may also be the case that z-SMA+ myofibroblasts contribute to renal injury through other mechanisms such as altering glomerular filtration or the production of growth factors which promote matrix production by glomerular and tubular epithelial cells.

We have a poor understanding of the mechanisms which initiate transdifferentiation and proliferation of myofibroblasts. Interstitial myofibroblasts express receptors for pro-fibrogenic cytokines such as transforming growth factor-β (TGF-β) and platelet-derived growth factor (PDGF) [13], TGF-β exerts a significant proliferative stimulus on myofibroblasts in culture [36], while administration of PDGF-BB to rats induced a dose-dependent proliferation of tubulointerstitial cells associated with marked expression of z-SMA [37]. In addition, upregulation of PDGF and TGF-β expression by renal tubules and interstitial cells has been described in areas of tubulointerstitial injury in the later stages following 5/6 nephrectomy [9,10]. These findings suggest an important role for PDGF-B and TGF-β in mediating myofibroblast transdifferentiation and/or proliferation.

In summary, this study has shown that local proliferation is an important mechanism in both macrophage and myofibroblast accumulation during the development of renal injury in the rat remnant kidney. In addition, local macrophage proliferation is postulated as a mechanism for amplifying kidney damage, irrespective of the immune or non-immune nature of the initial renal insult.
References


19. Damoiseaux JG, Dopp EA, Calame W, Chao D, MacPherson GG, Dijkstra C. Rat macrophage lysosomal membrane antigen recognized by monoclonal antibody ED1. Immunology 1994; 83: 140–147


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