Mast cells: the forgotten cells of renal fibrosis

I S D Roberts, P E C Brenchley

Abstract

Background/Aims—Mast cells, when activated, secrete a large number of fibrogenic factors and have been implicated in the development of fibrotic conditions of the liver, lung, and skin. There is evidence that renal fibrosis is closely linked with a chronic inflammatory cell infiltrate within the interstitium, but a potential role for mast cells in this process has yet to be defined. Therefore, the numbers of mast cells in normal and fibrotic kidneys with various pathologies were investigated.

Methods—Mast cells were quantified in renal transplants showing acute and chronic rejection and cyclosporin toxicity, kidneys removed for chronic pyelonephritis, and renal biopsies from patients with IgA nephropathy, membranous nephropathy, and diabetic nephropathy. Mast cells were stained using two methods: acid toluidine blue detected less than 30% of the mast cells revealed by immunohistochemistry for mast cell tryptase.

Results—Mast cells were scarce or absent in normal kidney (median, 1.6 mast cells/mm²) but numerous throughout the cortex and medulla in all specimens that showed fibrosis. They were almost entirely confined to the renal interstitium. Mast cells were present in large numbers in biopsies from patients with membranous nephropathy (median, 21.7 mast cells/mm²) and diabetic nephropathy (median, 29.2 mast cells/mm²), which were selected on the basis of showing chronic injury. In 24 unselected IgA nephropathy biopsies there was a close correlation between numbers of mast cells and the extent of interstitial fibrosis (r = 0.771; p < 0.0001). In renal transplant biopsies, mast cells were associated with allograft fibrosis in chronic rejection (median, 27.1 mast cells/mm²) and chronic cyclosporin toxicity (median, 10.6 mast cells/mm²) but not acute rejection (median, 2.7 mast cells/mm²) or acute cyclosporin toxicity (median, 2.0 mast cells/mm²). There was no detectable increase in mast cell numbers during acute rejection in those transplants that subsequently progressed to chronic rejection. In some biopsies the mast cells were largely intact, but in most cases some or all were degranulated.

Conclusions—An increased number of mast cells is a consistent feature of renal fibrosis, whatever the underlying pathology, and the number of mast cells correlates with the extent of interstitial fibrosis.

This suggests that mast cells might play a pathogenetic role in the fibrotic process.

Keywords: mast cells; kidney; fibrosis

Mast cells are a heterogenous group of cells, first identified by the dye binding characteristics of their proteoglycan rich granules. Their precursors originate from the bone marrow and circulate within peripheral blood; the mature tissue mast cells are widespread, being present in virtually every organ in the body. In addition to glycosaminoglycans, mast cells store, or are capable of synthesising, a wide variety of proinflammatory and vasoactive mediators, cytokines, and enzymes. The varied activity of mast cell products and their potential biological functions are multiple and diverse and they have been implicated in many pathological situations. In addition to type I hypersensitivity reactions there is evidence that mast cells play a role in acute inflammation, modulation of cellular immune responses, angiogenesis, turnover of connective tissue, and fibrosis.

Mast cells are known to secrete a range of cytokines including interleukin 3 (IL-3), IL-4, IL-5, IL-13, IL-6, and IL-8. Similarly, human mast cells constitutively express vascular endothelial growth factor (VEGF) and basic fibroblast growth factor (bFGF), which is localised to 97% of tryptase positive mast cells in human fibrotic lung tissue. Mast cells also contain specific proteases that display a range of biological functions. Mast cell tryptase is a mitogen both for epithelial cells, stimulating the production of IL-8 and intercellular adhesion molecule 1 (ICAM-1) expression, and for microvascular endothelial cells, inducing angiogenesis and capillary tube formation.

Chemoattractant factors controlling mast cell recruitment into inflammatory lesions have been identified as extracellular matrix components, including laminin, c-kit ligand (stem cell factor), platelet derived growth factor (PDGF), bFGF, VEGF, and the most potent of all, transforming growth factor β1 (TGF-β1). In peripheral lung tissue from patients with chronic obstructive pulmonary disease, associated with increased numbers of tissue mast cells, intraepithelial mast cell numbers correlate with TGF-β1 mRNA expression.

Mast cells have been implicated in pathological fibrosis in several conditions including hypertrophic scars, chronic atopic dermatitis, hepatic cirrhosis, fibrosing alveolitis, and cardiac fibrosis. In lung allograft rejection their numbers increase with the severity of acute rejection and the greatest numbers are associated with chronic rejection.
Mast cells in renal fibrosis

Mast cells in renal fibrosis might potentiate fibrosis by a variety of mechanisms. There is evidence that they are capable of synthesising several fibrogenic cytokines, including bFGF \(^{27}\) and TGF-\(\beta1\). \(^{28}\) Mast cell proteases might also contribute to matrix remodelling and fibrosis; in vitro studies have demonstrated activation of matrix metalloproteinas by mast cell degranulation. \(^{29}\) Mast cell tryptase is a profibrogenic factor, stimulating type I collagen synthesis by fibroblasts \(^{30}\) and inducing chemotaxis in fibroblasts comparable to that of TGF-\(\beta1\). \(^{31}\) Human mast cell chymase is effective at releasing latent TGF-\(\beta1\) from extracellular matrix but does not appear to convert TGF-\(\beta1\) to the active 25 kDa species. \(^{32}\) Furthermore, histamine and heparin control fibroblast proliferation and stimulate collagen synthesis in vitro. \(^{33}\) \(^{34}\) Conculture of mast cells and fibroblasts results in fibroblast proliferation, which is modulated by direct cell–cell contact. \(^{35}\) Mast cell granules are phagocytosed by fibroblasts in coculture \(^{36}\) and ultrastructural studies have confirmed an intimate association between mast cells and fibroblasts in vivo. The interaction between mast cells and stromal cells has been studied in several pathological tissues, including haemangiomas, \(^{37}\) spindle cell lipomas, \(^{38}\) and neurofibromas. \(^{39}\) Intercellular contacts, membrane fusion, and endocytotic vesicles have been described, and it is possible that direct cell–cell contact might be more important than mast cell degranulation or cytokine secretion in the control of connective tissue turnover.

Renal fibrosis is the major cause of end stage renal failure. Glomerulonephritis, diabetic nephropathy, pyelonephritis, and renovascular disease together account for over 75% of patients requiring renal replacement treatment. \(^{40}\) \(^{41}\) In these conditions, it is more commonly chronic progressive renal fibrosis, with associated loss of functioning nephrons, than a severe acute insult, that results in irreversible renal injury. There is abundant evidence that the long term outcome of chronic renal disease depends to a great extent upon changes within the tubulointerstitial compartment. This includes conditions in which the primary insult is within the glomeruli; the extent and severity of interstitial fibrosis and tubular atrophy are the most powerful histological markers of renal function and long term prognosis in chronic glomerulonephritis. \(^{42}\) \(^{43}\) \(^{44}\) Early on, this fibrotic process is characterised by an interstitial chronic inflammatory cell infiltrate and the proliferation of interstitial myofibroblasts. \(^{45}\) \(^{46}\) \(^{47}\) This is driven by a cytokine network that includes growth factors for myofibroblasts such as PDGF, bFGF, and TGF-\(\beta1\), an important trigger of matrix synthesis. \(^{48}\) The tubular epithelium is an important source of cytokines that act on interstitial fibroblasts \(^{49}\) and in addition stimulate the infiltration of T cells and macrophages, which then secrete several fibrogenic cytokines. \(^{50}\) \(^{51}\) The epithelial cells are also capable of transdifferentiation into myofibroblasts \(^{52}\) and elaborating matrix proteins. \(^{53}\) In chronic glomerulonephritis, cytokines secreted by glomerular epithelial cells, together with fibroblasts, play a role in producing tubular epithelial cell injury and activation, thus providing a major trigger for the fibroinflammatory response within the interstitium. \(^{54}\)

There is evidence that similar processes underlie the progressive interstitial fibrosis in chronic renal allograft rejection. Chronic rejection, the major cause of graft loss after renal transplantation, is characterised histologically by progressive vascular, glomerular, and interstitial fibrosis. As in native renal disease, TGF-\(\beta1\), secreted by tubular epithelium and infiltrating leucocytes, might play a central role in driving new matrix production. \(^{55}\)

Although there is an extensive literature on the role of lymphocytes and macrophages in the pathogenesis of renal fibrosis, a potential role of interstitial mast cells has been largely ignored. Other than within the matrix around the major vessels, mast cells are absent from normal kidney, but their role in renal disease is yet to be defined. Only recently has it been reported that mast cell infiltration is associated with chronic injury in diabetic nephropathy \(^{56}\) and IgA nephropathy. \(^{57}\) Here, we report the quantification of renal mast cell infiltrates in several fibrotic conditions. We demonstrate that increased numbers of mast cells is a consistent feature of renal disease, whatever the underlying pathology, and that the number of mast cells correlates with the extent of fibrosis.

**Methods**

**TISSUES**

Archival renal tissue, fixed in neutral buffered formalin (10% vol/vol) and embedded in paraffin wax, with the following diagnoses was used: “normal kidney” obtained from nephrectomy for renal cell carcinoma (\(n = 5\)); transplant nephrectomy for chronic vascular rejection (\(n = 5\)); nephrectomy for chronic obstructive pyelonephritis (\(n = 5\)); biopsies from renal transplants showing acute rejection (\(n = 19\)) and chronic vascular rejection (\(n = 8\)); acute cyclosporin toxicity (\(n = 13\)), and chronic cyclosporin toxicity (\(n = 12\)); and diagnostic renal biopsies from patients with IgA nephropathy (\(n = 24\)), membranous nephropathy (\(n = 4\)), and diabetic nephropathy (\(n = 6\)). The acute rejection transplant biopsies were consecutive and unselected from 19 patients. Of these, eight patients subsequently developed biopsy confirmed chronic rejection (group 1).

The eight biopsies showing chronic rejection were from this same group of patients. The remaining 11 patients (group 2) showed stable long term graft function. The IgA nephropathy biopsies were consecutive primary diagnoses over a two year period and were unselected. The membranous and diabetic nephropathy biopsies were selected on the basis of the haematoxylin and eosin sections showing mild to moderate chronic tubulointerstitial injury.

**QUANTIFICATION OF MAST CELLS**

The following two methods were used for the detection of mast cells in nephrectomy tissue:

1. 1% (wt/vol) toluidine blue, pH 1.0, staining time 30 minutes; and
2. Immunohistochemistry for mast cell tryptase (Dako Ltd, High

www.jclinpath.com
Wycombe, UK) using a standard immunoperoxidase technique. Sections were pretreated with hyaluronidase. The toluidine blue stain detected only a small proportion of the total mast cells present (table 1) and in all renal biopsies mast cells were detected immunohistochemically. In biopsy material the entire renal cortex was examined. In nephrectomy tissue mast cells were counted in 20 ×40 objective fields of the outer cortex (within two ×40 objective fields of the renal capsule), inner cortex (within two ×40 objective fields of the corticomedullary junction), and outer medulla. Numbers of mast cells/mm² were then calculated; each field had an area of 0.4 mm².

MORPHOMETRIC ANALYSIS

Biopsies on which the diagnosis of IgA nephropathy was made were analysed further to determine the extent of interstitial fibrosis. A Chalkley 25 point graticule (Graticules Ltd, Tonbridge, UK) was used to calculate the area of the interstitium, expressed as a percentage of the total renal cortical area. Periodic acid Schiff stained sections from each biopsy were used. Fields containing large arteries were excluded but otherwise the entire renal cortex in each biopsy was analysed. None of the biopsies showed pronounced oedema and, therefore, the interstitial area was a measure of the extent of interstitial fibrosis.

Results

NEPHRECTOMY SPECIMENS

In the “normal” renal tissue, obtained from kidneys that contained renal cell carcinomas, mast cells were present within the matrix around lobar and arcuate arteries, but absent from both the cortex and medulla, other than in small foci of subcapsular fibrosis. In contrast, large numbers of mast cells were present within the interstitium of kidneys showing chronic allograft rejection and chronic obstructive pyelonephritis (table 2; fig 1A and B). In chronic rejection, numbers of mast cells were generally greater in the cortex than in the medulla. There was no significant difference in numbers between the inner and outer cortex.

Table 1 Numbers of mast cells detected by toluidine blue compared with mast cell tryptase immunostaining

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>Mast cells/mm² of cortex median (range)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Toluidine blue (pH 1.0)</td>
</tr>
<tr>
<td>Normal kidney</td>
<td>0.2 (0–0.8)</td>
</tr>
<tr>
<td>CVR</td>
<td>12.5 (6.9–14.3)</td>
</tr>
<tr>
<td>CPN</td>
<td>7 (1.6–12)</td>
</tr>
</tbody>
</table>

p Values were calculated using the Mann-Whitney U test.

CVP, chronic obstructive pyelonephritis; CVR, chronic vascular rejection.

Table 2 Numbers of mast cells detected in nephrectomy specimens using immunohistochemistry for mast cell tryptase

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>Mast cells/mm² median (range)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Outer cortex</td>
</tr>
<tr>
<td>Normal kidney</td>
<td>2.5 (0.3–4.3)</td>
</tr>
<tr>
<td>CVR</td>
<td>35 (15.3–53.3)</td>
</tr>
<tr>
<td>CPN</td>
<td>30.5 (23.5–41.8)</td>
</tr>
</tbody>
</table>

CVR and CPN v normal kidney: p < 0.01 for cortex and medulla; CVR v CPN, not significant.

CVP, chronic obstructive pyelonephritis; CVR, chronic vascular rejection.

GLOMERULONEPHRITIS

Mast cells were present in large numbers within the interstitium in biopsies from patients with membranous nephropathy (median, 21.7 mast cells/mm²; range, 11.9–33.3) and diabetic nephropathy (median, 29.2 mast cells/mm²; range, 3.2–34.3). In the IgA nephropathy biopsies, there was a close correlation between numbers of mast cells and the extent of interstitial fibrosis (fig 2). In some biopsies the mast cells were largely intact, but in most cases some or all showed evidence of degranulation (fig 1C).

ALLOGRAFT BIOPSYSE

Mast cells were sparse in transplant biopsies showing acute rejection and acute cyclosporin toxicity (table 3). There was no difference in mast cell numbers between the acute rejection biopsies from group 1, patients who subsequently developed chronic vascular rejection, and those from group 2 with long term stable graft function. The subsequent biopsies...
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showing chronic rejection from group 2 patients contained large numbers of interstitial mast cells, as did the biopsies that showed chronic cyclosporin toxicity (table 3). Of the biopsies with chronic cyclosporin toxicity, five exhibited mild chronic allograft nephropathy (grade 1) and these contained fewer mast cells (median, 7.5/mm²) than the seven biopsies that showed chronic cyclosporin toxicity and chronic allograft nephropathy, grade 2–3 (median, 15.4/mm²), but this difference was not significant.

Discussion
We have demonstrated that an intratubular infiltrate of mast cells is a consistent feature of renal fibrosis, whatever the aetiology, and that the number of mast cells present correlates closely with the extent of fibrosis. The presence of mast cells is not confined to immune mediated or postinflammatory fibrosis, as indicated by increased numbers in chronic cyclosporin toxicity, in the absence of rejection.

The study of the role of mast cells in human pathology has been facilitated by the availability of antibodies to mast cell tryptase, an enzyme specific for mast cells, which can be used in formalin fixed tissues. The formalin sensitivity of traditional histochemical stains for mast cells, such as toluidine blue and alcian blue, has long been recognised and prevents the accurate quantification of mast cells in routinely processed tissue. Our initial quantification of toluidine blue stained mast cells in nephrectomy specimens indicates that around 75% of renal mast cells are formalin sensitive.

In renal allografts that show progression from acute to chronic rejection mast cells are not seen until the chronic phase, indicating that it is fibrosis itself, rather than the inflammatory process, that is the major stimulus for mast cell recruitment. It has been proposed that mast cells are a result, rather than a cause, of tissue fibrosis. However, in many of the renal tissues studied there is evidence of extensive mast cell degranulation. In view of the profibrotic properties of most cell products it appears likely that these cells play an active pathogenic role in the fibrotic process, rather than being merely innocent bystanders. Ehara et al reported bFGF production by interstitial mast cells in renal fibrosis associated with IgA nephropathy. We suggest that transiently increased TGF-β1 expression and activation in acute inflammation and immune responses is insufficient to establish a chemotactic gradient necessary to sustain mast cell influx because of competition for the bioactive TGF-β1 from resident fibroblasts, smooth muscle and endothelial cells, and infiltrating leucocytes. However, in a scenario of continued activation of TGF-β1 at the site of persistent chronic inflammation, high concentrations of bioactive TGF-β1 can bind to heparan sulphate glycosaminoglycan in the extracellular matrix and establish and sustain a haptotactic solid phase gradient, enabling mast cell recruitment. This mechanism is testable in models of TGF-β1 transfection with the use of selective neutralising anti-TGF-β1 antibodies.

Because many fibrogenic mast cell cytokines are also produced by other cell types involved in renal fibrosis, such as macrophages and tubular epithelial cells, it is difficult to define the precise role that mast cells play in the fibrotic process. One model that offers the potential for further investigation is the genetically mast cell deficient W/W mouse. If these animals show an impaired fibrogenic response after the induction of chronic renal injury that is not seen in the wild-type littermates with normal numbers of mast cells, and this is reversible after reconstitution with wild-type bone marrow cells, then an important role for mast cells in renal fibrosis is established. It has already been demonstrated in an animal model that cutaneous fibrosis may be inhibited by mast cell stabilising agents. If an active pathogenic role for mast cells in renal fibrosis is demonstrated, these compounds offer the potential for a new approach to antifibrotic treatment in the management of chronic renal disease.

Table 3 Numbers of mast cells in renal allograft biopsies

<table>
<thead>
<tr>
<th>Biopsy diagnosis</th>
<th>Mast cells/mm² of cortex median (interquartile range)</th>
<th>p Value *</th>
</tr>
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<tbody>
<tr>
<td>AR (no CVR)</td>
<td>3.0 (1.9–5.1)</td>
<td>AR vs CVR &lt;0.0001</td>
</tr>
<tr>
<td>AR (no CVR)</td>
<td>2.7 (1.0–5.3)</td>
<td></td>
</tr>
<tr>
<td>CVR</td>
<td>27.1 (20.7–29.8)</td>
<td></td>
</tr>
<tr>
<td>Acute cyclosporin toxicity</td>
<td>2.0 (1.3–2.5)</td>
<td>Acute vs chronic cyclosporin toxicity &lt;0.0001</td>
</tr>
<tr>
<td>Chronic cyclosporin toxicity</td>
<td>10.6 (7.2–21.3)</td>
<td></td>
</tr>
</tbody>
</table>

p Values were calculated using the Mann-Whitney U test.
AR, acute rejection; CPN, chronic obstructive pyelonephritis; CVR, chronic vascular rejection.

Figure 2 Correlation of numbers of mast cells with cortical interstitial area, expressed as a percentage of the total renal cortex. r = 0.771; p < 0.0001.

et al


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