Tubular kidney injury molecule-1 in protein-overload nephropathy

Mirjan M. van Timmeren,1 Stephan J. L. Bakker,2 Vishal S. Vaidya,3 Veronique Bailly,4 Theo A. Schuurs,5 Jeffrey Damman,5 Coen A. Stegeman,2 Joseph V. Bonventre,3 and Harry van Goor1

Departments of 1Pathology and Laboratory Medicine, 2Internal Medicine, and 3Surgery, University Medical Center Groningen and University of Groningen, Groningen, The Netherlands; 4Renal Division, Brigham and Women's Hospital, Harvard Medical School, Boston; and 4Biogen, Incorporated, Cambridge, Massachusetts

Submitted 13 October 2005; accepted in final form 1 February 2006

Van Timmeren, Mirjan M., Stephan J. L. Bakker, Vishal S. Vaidya, Veronique Bailly, Theo A. Schuurs, Jeffrey Damman, Coen A. Stegeman, Joseph V. Bonventre, and Harry van Goor.

Kidney injury molecule-1 (Kim-1) is a recently discovered membrane protein, which is undetectable in normal kidneys but markedly induced in proximal tubules after ischemic and toxic injury. The function of Kim-1 is unclear, but it is implicated in damage/repair processes. The Kim-1 ectodomain is cleaved by metalloproteinases and detectable in urine. We studied Kim-1 in a nontoxic, nonischemic, model of tubulointerstitial damage caused by acute proteinuria. Uninephrectomized (NX) rats received daily (ip) injections of 2 g BSA (NX + BSA, n = 12) or saline (NX, n = 6) for 3 wk. Kidneys were stained for various damage markers by immunohistochemistry (IHC). Kim-1 mRNA (RT-PCR, in situ hybridization), protein (IHC, Western blotting), and urinary Kim-1 (Luminex) were determined. Spatial relations between Kim-1 and other damage markers were studied by double labeling IHC. NX + BSA rats developed massive proteinuria (1,217 ± 313 vs. 18 ± 2 mg/day in NX, P < 0.001) and significant renal damage. Kim-1 mRNA was upregulated eightfold in NX + BSA (ratio Kim-1/β-actin, 4.08 ± 2.56 vs. 0.52 ± 0.64 in NX, P < 0.001) and localized to damaged tubules. Kim-1 protein expression was markedly induced in NX + BSA (2.46 ± 1.19 vs. 0.39 ± 0.10% staining/field in NX, P < 0.001). Urinary Kim-1 protein expression was markedly induced in NX + BSA (2.56 vs. 0.52 in NX, P < 0.001) and correlated with tissue Kim-1 expression (r = 0.66, P = 0.02). Kim-1 protein was found at the apical membrane of dilated nephrons. Kim-1 expression was limited to areas with inflammation (MÖ), fibrosis (α-smooth muscle actin), and tubular damage (osteonectin), and only occasionally with tubular dedifferentiation (vimentin). These results implicate involvement of Kim-1 in the pathogenesis of proteinuria-induced renal damage/repair. Urinary Kim-1 levels may serve as a marker of proteinuria-induced renal damage.

Kidney injury molecule-1 (Kim-1) is a recently discovered tubular protein that is markedly induced after ischemia-reperfusion injury and in response to a number of other proximal tubular toxins, including cisplatin and folic acid (10, 11). Kim-1 is a type I transmembrane glycoprotein, with an ectodomain containing an Ig-like domain and a mucin domain. In normal kidneys, Kim-1 is undetectable, but this molecule is abundantly expressed in PT after ischemic (10) and nephrotoxic injury (11). In mice, Kim-1 is upregulated in polycystic kidney disease especially in regions of the kidney where there is fibrosis (14). In humans and in rodents, Kim-1 is induced after ischemic or toxic acute tubular necrosis and the cleaved Kim-1 ectodomain is quantified in the urine as a biomarker for acute kidney injury (9, 21). This ectodomain shedding is confirmed in cultured human PTC where cleavage and shedding could be blocked by inhibition of metalloproteinase activity (1).

The early and abundant tubular expression following different types of injury makes Kim-1 a specific marker of tubular cell injury that may be linked to recuperative or damaging mechanisms that direct the process of interstitial damage, as has been proposed previously (14). Proteinuria has been proposed to cause tubular injury and interstitial fibrosis, therefore we studied Kim-1 expression in a nontoxic model of acute massive proteinuria, protein-overload nephropathy. In addition, we investigated whether urinary Kim-1 levels reflect renal tubular damage.

**MATERIALS AND METHODS**

**Experimental Design**

Protein-overload nephropathy was induced 1 wk after left nephrectomy in male Wistar rats (Harlan, Horst, The Netherlands), weighing 262 ± 13 g (means ± SD). Rats received intraperitoneal (ip) injections of 7 ml PBS (NX; n = 6) or 2 g BSA (low-endotoxin BSA, Sigma-Aldrich, Zwijndrecht, The Netherlands) dissolved in 7 ml PBS (NX + BSA; n = 12) six times a week for 3 consecutive weeks under isoflurane anesthesia. Rats were housed in a light- and temperature-controlled environment with free access to water and standard rat chow. Procedures were approved by the Committee for Animal

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
Experiments of the University of Groningen, and the Principles of Laboratory Animal Care (NIH publication no. 85–23) were followed. Body weight was measured weekly. Urine was collected weekly during a 24-h stay in metabolic cages with access to drinking water only. Tail vein blood samples were taken weekly under anesthesia. At the end of the study, rats were anesthetized, a blood sample was taken by cannulation of the aorta, and kidneys were perfused with saline. A coronal tissue slice was snap-frozen in isopentane and stored at −80°C. Another slice was fixed in 4% paraformaldehyde for paraffin embedding.

Measurements in Blood and Urine

Proteinuria was measured colorimetrically with the Pyrogallol-Red Molybdate method. Plasma and urine creatinine levels were determined by the Jaffe method (Merck, Darmstadt, Germany). Plasma triglycerides, cholesterol, and glucose were determined by the GPO-PAP, CHOD-PAP, and glucose dehydrogenase (Gluc-DH) methods (Merck, respectively).

Kim-1 protein in urine was measured by microsphere-based Luminex xMAP technology (20). This technique is an adaptation of the recently developed and validated sandwich ELISA assay (21). For measurements, 30 μl of 24-h urine were analyzed in duplicate.

RNA Extraction and RT-PCR

Total RNA was isolated from 10 slices of 10 μm frozen tissue with TRIzol. cDNA was synthesized from 2 μg RNA by oligo(dT) priming (1 h) using a Sensiscript Reverse Transcriptase kit (Qiagen/Westburg, Leusden, The Netherlands). PCR was performed as described (22) with gene-specific primer pairs for Kim-1 and β-actin: Kim-1 F, 5′-ACT CCT GCA GAC TGG AAT GG-3′; Kim-1 R, 5′-CAA AGC TCA CCA TC-3′; generating a 213-bp PCR product; β-actin F, 5′-AAC ACC CCA GCC ATG TAC G-3′; β-actin R, 5′-ATG TCA CGC AGC ATT CCC-3′; generating a 253-bp PCR product. Cycle conditions consisted of 40 s at 94°C, 40 s at 56°C, and 60 s at 72°C. The final extension was 7 min at 72°C. Each RNA sample was verified for the absence of genomic DNA by performing RT-PCR reactions without addition of an RT enzyme. The number of cycles verified for the absence of genomic DNA by performing RT-PCR was 72°C. The final extension was 7 min at 72°C. Each RNA sample was verified for the absence of genomic DNA by performing RT-PCR reactions without addition of an RT enzyme. The number of cycles verified for the absence of genomic DNA by performing RT-PCR was 34 for Kim-1, 24 for β-actin. Amplified products were analyzed by electrophoresis on 1.2% agarose gels and scanned on an Imagemaster VDS (Amersham Biosciences, Little Chalfont, UK) using LISCAP software. PCR product abundance was quantified using Imagemaster ID prime v3.00 (Amersham, Roosendaal, The Netherlands) and normalized for a β-actin signal from the same cDNA.

In Situ Hybridization

A 696-bp Kim-1 PCR product was formed with the primers Kim-1 F, 5′-AAC GCA GCG ATT GTG CAT CC-3′ and Kim-1 R, 5′-GTC CAC TCA CCA TGG TAA CC-3′ and cloned into the pCRII-TOPO vector (Invitrogen, Carlsbad, CA). DIG-labeled RNA probes were made with a DIG RNA labeling Kit (Sp6/T7, Roche, Mannheim, Germany). Deparaffinized sections were air-dried for 10 min and treated with Triton X-100, followed by proteinase K (5 μg/ml in PBS, Roche) at 37°C for 20 min. After being washed with PBS, slides were incubated with 10 ng/100 μl DIG-labeled probe (SP6/antisense or T7/sense) in hybridization solution consisting of 100 μl 50× Denhardt’s solution, 1 ml 20X SSC, 1 ml 50% dextran sulfate, 2.5 ml formamide, 200 μl (25 mg/ml) t-RNA, 49 μl 1 M DTT and 125 μl (10 mg/ml) salmon sperm DNA overnight at 55°C. After being washed, slides were treated with 2 U/ml RNase (Sigma-Aldrich, Steinheim, Germany) at 37°C for 30 min. Positive cells were visualized with anti-DIG-labeled alkaline phosphatase (Roche) for 1 h at 37°C in 0.1 M maleic acid buffer containing 0.15 M NaCl, 1% blocking buffer, and 2% normal sheep serum. Alkaline phosphatase reactivity was developed for 1 h at 37°C, followed by 24–48 h at 4°C, using NBT and BCIP (Roche) in 1 ml TBS containing 50 mM MgCl2 and 0.01 M levamisole.

Immunohistochemistry

Deparaffinized sections (4 μm) were subjected to heat-induced antigen retrieval by overnight incubation in 0.1 M Tris–HCl buffer (pH 9.0) at 80°C. Endogenous peroxidase was blocked for 30 min with 0.075% H2O2 in PBS. Primary antibodies (Table 1), diluted in 1% BSA/PBS, were incubated for 60 min at room temperature. Binding was detected using sequential incubations (30 min, Table 1) with appropriate peroxidase-labeled secondary antibodies (DakoCytomation) diluted in PBS with 1% BSA and 1% normal rat serum. Peroxidase activity was developed using 3,3′-diaminobenzidine tetrachloride (DAB) for 10 min. Sections were counterstained with periodic acid-Schiff (PAS) and hematoxylin. Appropriate isotype and PBS controls were consistently negative.

Measurement of renal damage. Focal glomerular sclerosis (FGS) was semiquantitatively scored (scale 0–4) in PAS-stained sections and expressed as the mean score of 50 glomeruli/kidney. FGS was

<table>
<thead>
<tr>
<th>Table 1. Primary antibodies used for immunohistochemistry</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Specificity</strong></td>
</tr>
<tr>
<td>-----------------</td>
</tr>
<tr>
<td>α-SMA</td>
</tr>
<tr>
<td>Macrophages</td>
</tr>
<tr>
<td>Desmin</td>
</tr>
<tr>
<td>Kim-1</td>
</tr>
<tr>
<td>Vimentin</td>
</tr>
<tr>
<td>Collagen III</td>
</tr>
<tr>
<td>Osteopontin</td>
</tr>
</tbody>
</table>

**Double-labeling**

| Vimentin       | Monoclonal mouse anti-vimentin clone V9 | DakoCytomation (Glostrup, Denmark) | 1:400 | GaM<sup>o</sup> |
| Kim-1          | Polyclonal rabbit anti-Kim-1 peptide 9 | V. Bailly | 1:400 | GaR<sup>o</sup> |
| α-SMA          | Monoclonal mouse anti-SMA clone 1A4 | Sigma (St. Louis, MO) | 1:5,000 | GaM<sup>o</sup> |
| Macrophages    | Monoclonal mouse anti-CD68, ED1 | Serotec Ltd. (Oxford, UK) | 1:250 | GaM<sup>o</sup> |
| Osteopontin    | Monoclonal mouse anti-MPIIIIB10 | Developmental Studies Hybridoma Bank (Baltimore, MD) | 1:500 | GaM<sup>o</sup> |

SMA, smooth muscle actin; Kim-1, kidney injury molecule-1; RoM<sup>o</sup>, peroxidase-conjugated rabbit anti-mouse antibody; GaR<sup>o</sup>, peroxidase-conjugated goat anti-rabbit antibody; RoG<sup>o</sup>, peroxidase-conjugated rabbit anti-goat antibody; GaM<sup>o</sup>, alkaline phosphatase-conjugated goat anti-mouse antibody.
Table 2. Clinical and pathological parameters at week 3

<table>
<thead>
<tr>
<th></th>
<th>NX (n = 6)</th>
<th>NX + BSA (n = 12)</th>
<th>P Value*</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Body weight, g</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total cholesterol, mmol/l</td>
<td>1.7±0.19</td>
<td>2.2±0.50*</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Fasting triglycerides, mmol/l</td>
<td>0.47±0.18</td>
<td>0.77±0.30*</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Fasting glucose, mmol/l</td>
<td>9.4±2.9</td>
<td>10.2±1.7</td>
<td></td>
</tr>
<tr>
<td>Total albumin, g/l</td>
<td>20.7±1.9</td>
<td>29.8±2.3†</td>
<td></td>
</tr>
<tr>
<td><strong>Creatinine clearance, ml/min</strong></td>
<td>18±2</td>
<td>1,217±313†</td>
<td></td>
</tr>
<tr>
<td><strong>Creatinine clearance, mg/day</strong></td>
<td>1.5±0.12</td>
<td>1.4±0.47</td>
<td></td>
</tr>
</tbody>
</table>

Values are means ± SD. n, No. of rats; NX, uninephrectomized control rats; NX+BSA, protein-overload rats. *P < 0.05 and †P < 0.001 vs. NX by t-test.

Table 3. Renal damage parameters at week 3

<table>
<thead>
<tr>
<th></th>
<th>NX (n = 6)</th>
<th>NX + BSA (n = 12)</th>
<th>P Value†</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Glomerular damage</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FGS score (scale 0–4)</td>
<td>0.04±0.06</td>
<td>1.13±0.32</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>M0, no.</td>
<td>3.7±2.0</td>
<td>15.7±4.8</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Desmin score (scale 0–4)</td>
<td>0.7±0.5</td>
<td>3.4±0.2</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>α-SMA, %area</td>
<td>1.8±0.5</td>
<td>10.2±3.6</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>Interstitial damage</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M0, no.</td>
<td>27.7±12.0</td>
<td>60.0±21.7</td>
<td>0.006</td>
</tr>
<tr>
<td>Collagen III, %area</td>
<td>8.4±2.3</td>
<td>14.1±3.5</td>
<td>0.003</td>
</tr>
<tr>
<td>Osteopontin, %area</td>
<td>0.12±0.09</td>
<td>3.60±1.78</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>α-SMA, %area</td>
<td>1.09±0.34</td>
<td>9.70±3.30</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Values are means ± SD. n, No. of rats; FGS, focal glomerular sclerosis; M0, macrophages; *NX + BSA vs. NX, t-test. †Mean of 50 glomeruli. ‡Mean of 30 cortical fields.

RESULTS

Protein Overload Characteristics

Clinical and pathological parameters are presented in Table 2. All NX + BSA rats developed massive proteinuria compared with NX (1.217 ± 313 vs. 18 ± 2 mg/day, P < 0.001). Kidney weight was almost doubled in NX + BSA compared with NX rats (4.1 ± 0.7 vs. 2.3 ± 0.3 g, P < 0.001), whereas total body weight did not differ. Plasma albumin, cholesterol, and triglycerides were elevated in NX + BSA vs. NX rats; plasma glucose and creatinine clearance did not differ between the two groups.

All NX + BSA rats revealed marked renal damage compared with NX rats receiving PBS (Table 3). In NX + BSA, mild FGS was found with massively increased glomerular visceral epithelial desmin expression. In the tubulointerstitium, focal areas of tubular dilatation and fibrosis were present. M0 influx was significantly increased in glomeruli and tubulointerstitium. Interstitial fibrosis in NX + BSA rats was evidenced by excessive amounts of extracellular matrix (collagen III) and an increased presence of myofibroblasts (α-SMA). Also, tubular OPN was markedly induced in NX + BSA.

Kim-1 mRNA

Semi-quantitative RT-PCR revealed low levels of Kim-1 mRNA in the kidneys removed at nephrectomy (i.e., before the study) and in saline-injected NX kidneys (Fig. 1). In NX + BSA Kim-1 mRNA levels were upregulated eightfold compared...
Kim-1 mRNA was markedly upregulated in NX kidneys (both \( P < 0.001 \)). Using in situ hybridization, traces of Kim-1 mRNA were found in a cluster of morphologically unaffected cortical tubules of NX kidneys (Fig. 2A). In contrast, in NX+BSA rats Kim-1 mRNA was abundantly expressed in a typical cytoplasmic staining pattern in diluted tubules in fibrotic areas throughout the entire cortex (Fig. 2B). In addition, some morphologically intact cortical proximal tubules were Kim-1 positive as well. Kim-1 was often present in a heterogeneous expression pattern within one cross-sectional tubular structure, with Kim-1-positive and -negative tubular cells adjacent to each other (Fig. 2C). Dilated tubules with fully denuded tubular membranes and shrunken cells were Kim-1 negative. Occasionally, tubular structures in the medulla showed Kim-1 mRNA expression.

**Kim-1 Immunohistochemistry**

Kim-1 protein expression was undetectable in the kidneys removed at nephrectomy before the study, whereas immunohistochemistry sporadically revealed Kim-1-positive cortical tubules in NX rats (Fig. 2E). Kim-1 protein expression was massively upregulated in NX+BSA compared with NX rats (2.33 ± 1.07 vs. 0.39 ± 0.11% staining/field, \( P < 0.001 \); Fig. 1B). In NX+BSA kidneys, Kim-1 protein expression was predominantly located in the outer medulla, with focal cortical extensions. Single dilated nephron segments with flattened epithelium from outer medulla to the outer cortex were often positive (Fig. 2F). Protein expression was localized to the apical part of dilated tubules in fibrotic areas, with occasionally diffuse cytoplasmic staining. Kim-1-expressing dilated tubules often possessed a thickened or disrupted basement membrane and no visible cellular brush border. Besides Kim-1 expression in diluted tubules, less pronounced, primarily apical, Kim-1 expression was found in some morphologically intact proximal tubules. Consistent with the in situ hybridization results, we often noted a heterogeneous Kim-1-staining pattern between individual cells within one cross-sectional dilated tubular structure (Fig. 2G). The renal papilla, glomeruli, and tubular casts were negative.

**Kim-1 Western Blotting**

For further quantifications of Kim-1 protein expression after protein overload, membrane fractions of kidney homogenates from NX and NX+BSA were analyzed. In NX+BSA rats, besides a 50-kDa protein that was also present in NX, two additional protein bands were seen with a molecular mass of ~40 and 70–80 kDa (Fig. 3), corresponding to previous results by Ichimura et al. (10). These bands possibly represent different degrees of glycosylation (10).

**Urinary Kim-1**

At baseline, low levels of urinary Kim-1 protein were found. After protein-overload induction, urinary Kim-1 was significantly higher in NX+BSA than in NX controls at all measured time points (Fig. 4). The amount of urinary Kim-1 at week 3 correlated with Kim-1 tissue expression \(( r = 0.66, P = 0.02)\) and proteinuria \(( r = 0.63, P = 0.04)\).

**Characteristics of Kim-1 Expression**

*Kim-1 related to tubulointerstitial damage.* To characterize tubular Kim-1 expression in relation to classic markers of interstitial inflammation and fibrosis, we compared Kim-1 expression to MØ infiltration and α-SMA expression. Double-labeling immunohistochemistry revealed that Kim-1-positive tubules were associated with aggregates of MØ and areas with increased α-SMA expression (Fig. 5, A and C). There was a clear correlation between Kim-1 expression and interstitial MØ \(( r = 0.95, P < 0.001)\) and interstitial α-SMA \(( r = 0.67, P = 0.001; \text{Fig. } 5, B \text{ and } D)\).

*Kim-1 related to tubular cell damage but not vimentin staining.* We characterized Kim-1-expressing tubular cells by double labeling with OPN (tubular marker of chemotaxis and repair) and vimentin (marker of tubular dedifferentiation).

Double labeling revealed a spatial relationship between OPN and Kim-1. In tubulointerstitial fibrotic areas, virtually all dilated tubules expressing Kim-1 were also OPN positive (Fig. 5E). In these tubules, Kim-1 is expressed at the apical membrane whereas OPN expression is predominantly cytoplasmatic (Fig. 5F). Furthermore, OPN but not Kim-1 expression was found in morphologically unaffected tubules directly surrounding Kim-1/OPN double-positive tubules. A clear correlation between tubular Kim-1 and OPN expression was found \(( r = 0.92, P < 0.001)\).

Double labeling for Kim-1 and vimentin revealed a mosaic tubular staining pattern in areas with tubulointerstitial damage. Vimentin was expressed in extremely damaged tubular structures at the luminal and lateral side of the cell. However, only a few tubular cells expressed both Kim-1 and vimentin, and tubular structures with less pronounced damage expressed Kim-1 only (Fig. 5, G and H).
DISCUSSION

The major finding of this study is a marked induction of tubular Kim-1 and urinary Kim-1 shedding during acute proteinuria. Tubular Kim-1 is predominantly expressed in areas with tubulointerstitial damage, characterized by the presence of MØ, tubular dilatation, and fibrosis. Kim-1 displays a heterogeneous expression pattern within one tubular cross section. Based on morphological data, we propose that tubular Kim-1 follows and largely coincides with OPN and precedes vimentin-associated epithelial dedifferentiation.

In this study, we describe Kim-1 in acute proteinuria-induced renal damage. Kim-1 induction has been demonstrated...
Kim-1 induction in proteinuric renal damage

After ischemic (10, 18) and nephrototoxic (11) renal injury, in patients with acute ischemic tubular necrosis (9), and in renal cell carcinoma (8). In agreement with studies in ischemia-reperfusion (10, 18) Kim-1 was predominantly expressed in dilated and flattened cortical tubules. Similar to other studies (9–11), we primarily found Kim-1 localized to the apical part of the cell with occasionally a cytoplasmic staining pattern. The mosaic staining pattern within one tubular cross section resembles Kim-1 expression after folic acid- and cisplatin-induced renal injury (11) and in cystic kidneys (14), although it was absent after transient ureteral obstruction (14). As in nephrototoxic renal injury (11), ischemic acute tubular necrosis (9), and renal cell carcinoma (8), we also found high urinary Kim-1 after proteinuria-induced renal damage. Moreover, urinary Kim-1 levels during acute proteinuria correlated with Kim-1 tissue expression.

Although the present study does not provide evidence on the trigger(s) for Kim-1 induction, various mechanisms could be involved. First, protein-overload nephropathy is associated with activation of PTC by a tubulotoxic ultrafiltrate, and subsequent production of proinflammatory and profibrotic mediators, which initiate tubulointerstitial damage (4, 5, 7). PTC in vitro exposed to high concentrations of proteins present in the ultrafiltrate of nephrotic patients, show increased synthesis of cytokines/chemokines, such as MCP-1, RANTES, IL-8, endothelin-1, and TGF-β (27). Kim-1 induction might well be part of that general pathway leading from initial tubular activation to chronic damage caused by extended exposure to the tubulotoxic ultrafiltrate.

In addition to the role of the tubulotoxic ultrafiltrate, lack of oxygen might also be involved in Kim-1 induction. Kim-1 expression was first noted in rats after ischemic injury in corticomedullary areas, the most vulnerable site for oxygen deprivation (10), and in patients with ischemic acute tubular necrosis (9), which led these authors to suggest that ischemia/hypoxia is a major trigger for Kim-1 induction. However, in murine polycystic kidney disease, Kim-1 is upregulated without signs of ischemic injury (14). In our model, tubular protein overloading could lead to loss of tubulointerstitial perfusion caused by edema or loss of peritubular capillaries (3). Cytokines and growth factors produced by activated tubular cells cause interstitial changes such as fibrosis and MØ accumulation. This process may cause damage to the capillary endothelium and/or activate interstitial fibroblasts to produce extracellular matrix. These local processes may hamper oxygen supply to adjacent tubular cells, known to be extremely vulnerable to oxygen deprivation. Furthermore, a tubular disbalance in energy expenditure and availability might also result in tubular ischemia, because tubular protein overloading leads to an increased lysosomal processing (13), affecting oxygen demand.

The third possible mechanism for induction of Kim-1 is tubular obstruction by cast formation. In protein overload, the formation of protein casts and the subsequent tubular obstruction, mechanical stress, and increased glomerular pressure may activate tubular cells, leading to Kim-1 induction. Kim-1 structurally resembles mucosal addressin cell adhesion molecule-1 (1). It is possible that Kim-1 is involved in surface adhesion interactions and possibly cast formation. However, in our model tubular casts were not related to Kim-1 expression, as was seen in nephrotic injury (11), which makes it unlikely that cast formation only triggers Kim-1 induction. As Kim-1 expression is seen in different situations and models, it is most likely that induction of Kim-1 is dependent on an interplay of different factors, leading to tubular cell stress and activation.

OPN, vimentin, and Kim-1 showed a focal expression pattern, indicating that not all nephrons are damaged to the same extent. Apparently, nephrons are unequally vulnerable to damage and/or exposed to unequal amounts of tubulotoxic ultrafiltrate. Also, cells may respond in different ways to injury: uninjured cells proliferate and repopulate damaged nephron segments with new cells. Nonlethally damaged cells recover, and some damaged cells may undergo cell death by apoptosis or necrosis (19). Because all different stages are present after injury, this probably explains the focal distribution of damage and the heterogeneous Kim-1 expression between and within tubular structures.

In response to injury, tubular cells express various proteins. We found OPN-positive, morphologically unaffected, tubular...
structures that did not stain for Kim-1 surrounding areas with severe fibrosis and coexpression of tubular OPN and Kim-1. This suggests that in early stages of damage OPN expression may precede Kim-1 expression. After OPN and Kim-1 expression, the PTC may either undergo apoptosis/necrosis or develop into a nonmature, dedifferentiated vimentin-positive tubular cell. Dedifferentiated vimentin-positive cells can proliferate and restore the tubular structure (2). It has also been proposed that these cells can transform from an epithelial to mesenchymal cell type (EMT) and migrate as fibroblasts into the tubulointerstitium and contribute to fibrosis (12). Thus vimentin-positive cells are important in restoration of tubular

Fig. 5. Double-labeling immunohistochemistry in NX+/BSA rats. A: Kim-1-positive tubules (brown) are associated with aggregates of infiltrating macrophages (MØ; blue). C: Kim-1 expression (brown) is associated with fibrosis [α-smooth muscle actin (α-SMA) expression in blue]. A and C are serial sections. B and D: correlation between Kim-1 expression and MØ (B) and Kim-1 and α-SMA expression (D) in NX+/BSA rat kidneys (●). NX rats (○) were not included in the correlation. E: Kim-1 (brown) and osteopontin (OPN; blue) are expressed in the same tubules, although there are some OPN-positive tubules that do not express Kim-1. These are seen surrounding Kim-1/OPN-double-positive tubules. F: detail of Kim-1 and OPN staining; Kim-1 expression is at the apical membrane, whereas OPN expression is cytoplasmic. G: tubular Kim-1 (brown) and vimentin (blue) expression are found in fibrotic areas. Note the mosaic staining pattern for Kim-1 and vimentin within one tubular cross section; only few tubular cells express both Kim-1 and vimentin (H). Original magnification: ×200 (A, C, E, and G); ×630 (F); ×400 (H).
structures. Kim-1 colocalized with vimentin after ischemia-reperfusion (10) and S-(1,1,2,2-tetrafluoroethyl)-t-cysteine-induced renal injury (11). After folic acid- and cisplatin-induced injury, Kim-1 was expressed in the absence of detectable vimentin staining, whereas in a later phase coexpression of Kim-1 and vimentin increased (11). In our study, we only found scarce colocalization, with vimentin predominantly expressed in more damaged and Kim-1 in less damaged tubular areas. This may suggest a sequential or damage-dependent expression of these two markers, which can only be proved by sequential analyses in times earlier in the model.

Kim-1 is induced after various types of injury, and both tissue and urinary Kim-1 expression can serve as markers for renal damage (9, 11). However, the function of Kim-1 remains unclear. Kim-1 might either actively modulate tubulointerstitial injury as has been proposed (14) or be a consequence of tubulointerstitial injury. Structurally, Kim-1 resembles an adhesion molecule, and human Kim-1 is also known as hepatitis A virus cellular receptor (6, 23) and T cell immunoglobulin mucin-like domain 1 (TIM-1) (15, 17). TIM-1 is expressed on T cells, and in mice it was demonstrated that Tim-4 is the natural ligand for Tim-1 (16). This suggests that Kim-1 might also interact with other protein(s). Intriguingly, the extracellular part of Kim-1 can be shed into the tubular lumen, and this ectodomain shedding is metalloproteinase driven (26). In vivo the Kim-1 ectodomain may form a protective layer on the proximal tubular cells, thereby protecting them from protein casts that are formed within the tubular lumen. Kim-1 may also prevent the formation of tubular casts. Although the pathophysiological implications of Kim-1 shedding are unclear, it can be used as a urinary biomarker (8, 9).

In conclusion, Kim-1 is markedly induced in proteinuric renal disease. Expression is largely restricted to tubular cells in areas with tubulointerstitial damage, suggesting a modulatory role in renal damage. Based on its colocalization with OPN, but limited association with epithelial dedifferentiation, we hypothesize that Kim-1 induction is an intermediate step in the sequence of events progressing from primary tubular activation to end-stage tubulointerstitial damage. Urinary Kim-1 levels correlate with renal Kim-1 expression, and thus Kim-1 may be used as a relevant biomarker for proteinuria-induced renal damage. The exact functional role of Kim-1 in proteinuria-induced tubulointerstitial damage remains to be elucidated.

ACKNOWLEDGMENTS

We are grateful to P. A. Klok, M. Bulthuis, and S. Huitema for excellent technical assistance. These data were presented at the 38th Annual Meeting of the American Society of Nephrology, Philadelphia, PA, November 8–13, 2005.

GRANTS

This work was supported by a grant from the Graduate School, Groningen University Institute for Drug Exploration (GUIDE). J. V. Bonventre was supported by National Institute of Diabetes and Digestive and Kidney Diseases Grants DK-39773 and DK-72381. V. S. Vaidya was supported by Scientist Development Grant 0535492T from the American Heart Association.

DISCLOSURES

None of the authors has any conflict of interest.

REFERENCES


