Nephrol Dial Transplant (1997) 12: 1344–1353

Nephrology
Dialysis
Transplantation

Original Article

Upregulated renal tubular CD44, hyaluronan, and osteopontin in *kdkd* mice with interstitial nephritis

V. Sibalic[1], X. Fan[1], J. Loffing[2] and R. P. Wüthrich[1,3]

Institutes of [1]Physiology and [2]Anatomy, University of Zürich-Irchel, and [3]Division of Nephrology, University Hospital, Zürich, Switzerland

Abstract

Background. The hyaluronan (HA) receptor CD44 is upregulated on parenchymal cells in various inflammatory lesions and could play a role in immune injury. The purpose of the present study was to examine CD44 and its ligands HA and osteopontin (Opn) in a murine model of tubulointerstitial nephritis (TIN).

Methods. The expression of CD44 was investigated by immunofluorescence staining and RNA analysis in kidneys of *kdkd* mice with autoimmune TIN. The CD44 expression was then correlated with the location of its ligands HA and Opn.

Results. CD44 is expressed *de novo* by tubular epithelial cells (TEC) in areas of tubular injury in *kdkd* kidneys, but not in normal control kidneys. CD44 positive lymphocytes and macrophages also infiltrate the kidney in *kdkd* mice. RT-PCR and Southern blot analysis demonstrate that transcripts encoding standard and variant forms of CD44 are increased in *kdkd* mice with TIN. In parallel the CD44 ligand HA also accumulates in *kdkd* kidneys in the interstitial space, particularly in cortical areas of tubular injury. Furthermore, the expression of the chemotactic protein Opn is enhanced in *kdkd* kidney, predominantly in areas of tubular injury. Opn mRNA expression also increases markedly in *kdkd* kidneys compared with normal kidneys, and correlates with disease severity.

Conclusions. Prominent CD44 expression by TEC in areas of tubulointerstitial lesions is a characteristic feature of *kdkd* mice. The *de novo* appearance of CD44 on injured TEC might allow interaction with the ligands HA and Opn *in vivo*. Interaction of CD44 with these ligands could participate in the tubulointerstitial inflammatory response in *kdkd* mice.

Key words: CD44; hyaluronan; interstitial nephritis; *kdkd*; osteopontin

Correspondence and offprint requests to: Rudolf P. Wüthrich MD, Division of Nephrology, University Hospital, Rämistrasse 100, 8091 Zürich, Switzerland.

Introduction

Mutant CBA/CaH mice, which are homozygous at the *kd* locus on chromosome 10, develop a progressive T-cell-mediated tubulointerstitial nephritis (TIN) [1,2]. The molecular nature of the genetic defect is presently unknown. CBA/CaH-*kdkd* mice develop characteristic lesions in the renal cortex early in life, consisting of focal peritubular mononuclear infiltration and tubular dilatation that progressively involves the entire cortex [1,3]. Mice usually die between 5 and 7 months of end-stage renal failure.

The pathogenesis of the nephritic process in *kdkd* mice is incompletely understood. Because of the tubular dilatation it was originally felt that the *kd* mutation could represent the murine homologue of juvenile nephronophthisis [1]. However, later studies have convincingly demonstrated that the disease is an autoimmune disorder [3–6]. TIN in *kdkd* mice is primarily mediated by pathogenic CD8+ T cells that recognize renal tubular epithelial antigens [5]. Downregulation of suppressor cells could also contribute to the nephritic process in *kdkd* mice [4]. Autoantibodies targeting tubulointerstitial determinants are not involved [3].

We have previously reported that the hyaluronan receptor CD44 is expressed *de novo* on tubular epithelial cells (TEC) in MRL-*Fas*^{lpr} mice with lupus nephritis [7]. Others have reported on the enhanced expression of CD44 by proximal TEC in human renal biopsies from patients with immune-mediated diseases such as SLE and IgA nephritis [8]. The functional significance of CD44 induction on TEC is presently unclear. The purpose of the present study was to examine renal CD44 expression in a disease model where tubulointerstitial disease is the primary pathogenic event, namely the *kdkd* mouse model of TIN. Furthermore we correlated CD44 with the expression of its natural ligands HA (a matrix constituent) and the chemotactic cytokine Opn. Here we demonstrate that CD44 is expressed *de novo* by renal TEC in areas of tubular injury in *kdkd* kidney. Furthermore, both HA and Opn are enhanced in *kdkd* kidneys and colocalize with CD44 on injured TEC. Interaction of

CD44 with these ligands could alter TEC function and modulate the tubulointerstitial inflammatory response in *kdkd* nephritis. HA and Opn may have additional functions in *kdkd* TIN, such as tissue oedema formation and chemotaxis respectively.

Subjects and methods

Mice

Normal CBA/CaH and mutant CBA/CaH-*kd* (*kdkd*) mice were obtained from Jackson Laboratories (Bar Harbor, ME, USA) and were fed standard laboratory chow. Mice were killed at various ages and kidneys were processed immediately for RNA extraction.

Indirect immunofluorescence staining

Mice were anaesthetized with thiopental and kidneys were then fixed by intravascular perfusion with high pressure (approx. 250 mmHg) through the abdominal aorta. The fixative solution contained 3% paraformaldehyde (w/v), 0.05% picric acid (w/v) and 0.1 M cacodylate buffer (pH 7.4, adjusted to 300 mOsm with sucrose). After 3 min of perfusion, kidneys were removed and frozen in liquid propane that was cooled with liquid nitrogen. The tissue was stored at -70°C until use.

Cryostat sections (5 μm) were transferred on poly-L-lysine-coated slides (100 μg/ml) and were stained for CD44, HA and Opn by indirect immunofluorescence. For optimal CD44 staining a combination of three mAb culture supernatants was used, including rat anti-mouse mAbs IM7.8.1 [9], KM201 [10] and IRAWB 14 [11]. To detect HA we used a biotinylated proteoglycan (b-PG) probe that specifically recognizes HA (kindly provided by Dr Charles Underhill, Washington, DC, USA) [12]. Opn was detected by using a specific rabbit antiserum (kindly provided by Dr Elaine Worcester, Milwaukee, WI, USA) [13,14]. Sections were first blocked with 3% milk powder for 15 min. Sections were then incubated with primary antibody or b-PG for 2 h, were washed with PBS, and were incubated with FITC-conjugated goat anti-rat IgG (Sigma Chemical Co., St Louis, MO, USA) (for CD44), streptavidin-Cy3 (Sigma) (for b-PG) or Cy3-conjugated goat anti-rabbit IgG (Jackson Immuno Research Laboratories, West Grove, PA) (for Opn) for 45 min. Sections were then rinsed, mounted and examined by fluorescence microscopy. Controls included omission of primary antibody and staining with irrelevant antibodies. Pretreatment with hyaluronidase was used to demonstrate specificity of HA staining with the b-PG probe.

Tissue RNA extraction

Total RNA was isolated from normal and *kdkd* kidneys according to Chirgwin *et al.* [15]. Tissues were homogenized in a 4 M guanidinium isothiocyanate solution and ultracentrifuged at 35 000 r.p.m. through a 5.7 M $CsCl_2$ layer. Pellets were washed with 70% ethanol, dissolved in DEPC-treated H_2O, and quantitated by measuring the OD at 260 nm.

Semiquantitative RT-PCR

CD44 gene expression was assessed by semiquantitative RT-PCR using a kit (Perkin–Elmer, Branchburg, NJ, USA). The following primers were used: CD44 sense 5′-ACC CCA GAA GGC TAC ATT TTG C-3′, CD44 anti-sense 5′-CTC ATA GGA CCA GAA GTT GTG G-3′ (221 bp fragment) [16,17]. These primers directly flank the alternative splicing site where variant exons can be inserted, allowing convenient assessment for the presence of variant forms of CD44. The housekeeping gene GAPDH was used to control for equal amounts of RNA as described [7]. One-microgram aliquots of total kidney RNA were reverse transcribed. Following extension at 42°C for 45 min the reverse transcriptase was inactivated at 95°C for 5 min. PCR was then performed, using the following cycling parameters: denaturation at 94°C for 40 s, annealing at 55°C for 120 s, extension at 72°C for 150 s over 40 cycles. RT-PCR products were resolved on 1% agarose gels in 1 × TBE buffer. Gels were stained with 0.5 μg/ml ethidium bromide and photographed under UV light.

Southern blotting

To ensure specificity of the amplified fragments we performed Southern blot analysis and hybridization with a recombinant cDNA encoding CD44. Gels were denatured with 0.5 N NaOH in 1 M NaCl for 30 min, were neutralized with 0.5 M Tris–HCl (pH 7.4) in 3 M NaCl for 30 min and were transferred onto nylon membranes (Zeta-Probe blotting membranes, BioRad Laboratories, Hercules, CA), using 10 × SSC. Blots were then prehybridized with 100 μg/ml sheared, denatured salmon sperm DNA. The plasmid containing a 1.1 kb murine CD44 *Eco*RI cDNA fragment was obtained from Dr E. Butcher (Palo Alto, CA) [17]. The cDNA fragment was radiolabelled using random hexamers and α-^{32}P dCTP [18]. Blots were hybridized overnight at 42°C in 40% formamide, 6 × SSC, 5 × Denhardt's solution and 1% SDS. Blots were then washed under stringent conditions (final wash in 0.2 × SSC, 1% SDS, 60°C). Following hybridization the blots were exposed to Kodak X-OMAT AR film.

Northern blotting

Northern blotting was used to detect Opn transcripts in normal and *kdkd* kidneys. Total RNA (25 μg) was electrophoresed on 1% agarose gels in 20 mM MOPS buffer and blotted on nylon membranes. Membranes were prehybridized with salmon sperm DNA (10 μg/ml) for 6 h and were then hybridized overnight with a ^{32}P-labelled Opn cDNA probe. The Opn probe was obtained by RT-PCR amplification of RNA derived from normal mouse kidney, using primers located at position 169–188 and 855–874 of the published sequence [19], yielding a 706 bp cDNA fragment. After stringent washes, blots were exposed to Kodak X-OMAT AR film. Blots were then rehybridized with a ^{32}P-labelled cDNA probe for the housekeeping gene GAPDH that was also derived by RT-PCR. Densitometric analysis of the autoradiographs was performed with the ImageQuaNT® software (Molecular Dynamics, Sunnyvale, CA).

Results

Enhanced tubular CD44 expression in kdkd *mice*

Normal and *kdkd* mice were examined at various disease stages for CD44 expression by immunofluorescence staining (Figure 1). In normal CBA/CaH and young *kdkd* mice staining for CD44 was limited to a few mononuclear interstitial cells (Figure 1A). Except

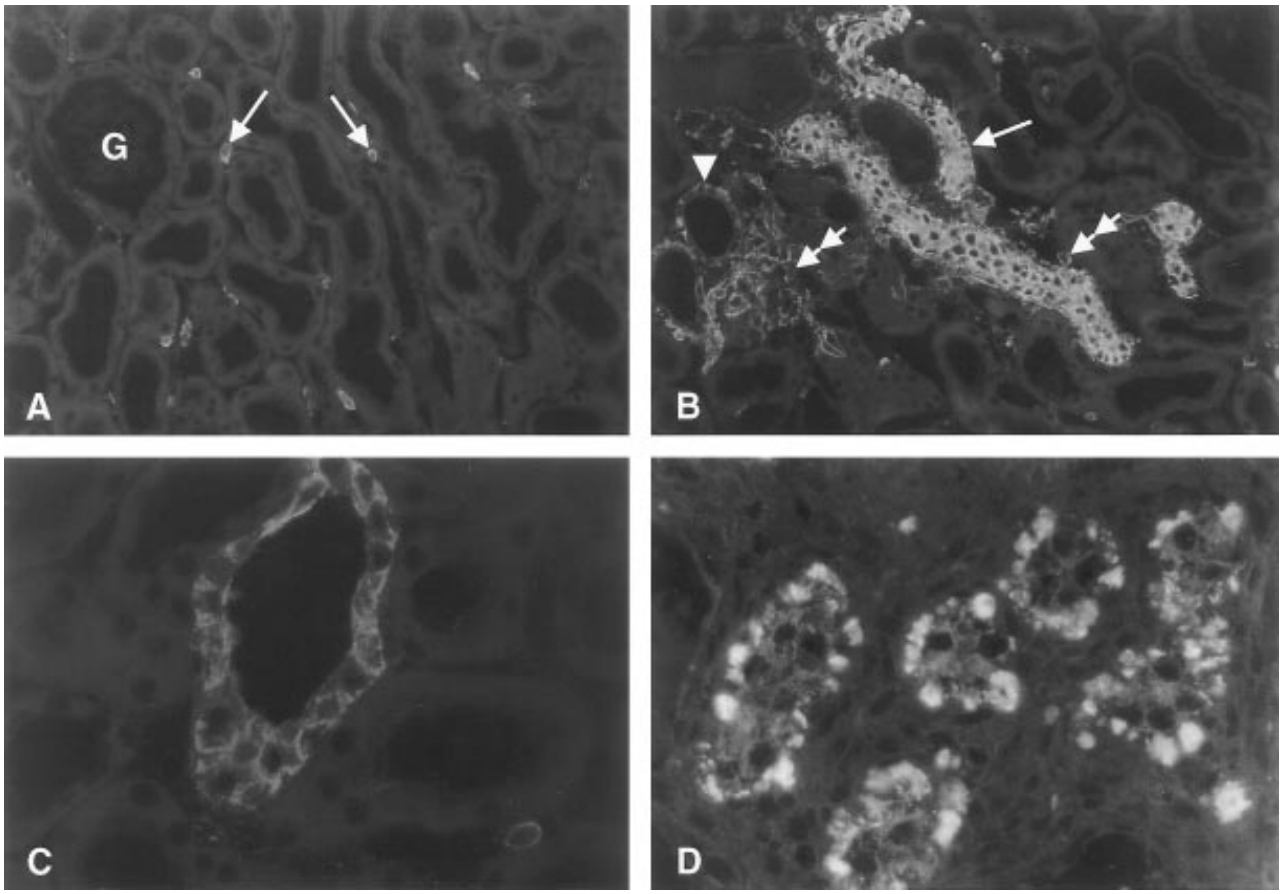

Fig. 1. Immunofluorescence staining for CD44 in 4-month-old normal control CBA/CaH (A) and *kdkd* mice (B-D). **A**, CBA/CaH mouse. The tubules appear normal in shape with an open lumen and negative staining for CD44. No sclerosis, cellular infiltration, or mesangial hypercellularity is detected in the glomeruli (G). The interstitium has no sign of inflammation. A few interstitial cells are CD44 positive (arrows) (magnification × 400). **B**, *Kdkd* mouse. Collapsed proximal tubules stain strongly for CD44 (arrow), whereas non-collapsed altered tubules display weaker CD44 (arrowhead). Note the CD44 positive infiltrating cells (double arrow) (× 400). **C**, *Kdkd* mouse. Non-collapsed, isolated CD44 positive tubule (× 1000). **D**, *Kdkd* mouse. Severely altered and collapsed cortical tubules display strong granular CD44 staining towards the interstitium (× 1000).

that the macula densa was weakly stained for CD44 basally (not shown) no staining was detected in glomeruli and tubules. With progression of the tubular lesions CD44 appeared *de novo* on TEC in the renal cortex of *kdkd* mice (Figure 1B). Furthermore, infiltrating mononuclear leukocytes in areas of tubular injury were also CD44 positive (Figure 1B). Immunophenotyping showed that these mononuclear cells were lymphocytes and macrophages (not shown). Non-collapsed tubules with altered morphology (dense nuclear arrangement and a low cytoplasm/nucleus ratio) expressed CD44 basolaterally and apically (Figure 1C). Staining was most prominent on severely injured and collapsed tubules in a focal pattern throughout the cortex. Fine granular deposits staining intensely for CD44 were present on the basolateral side of TEC, and larger deposits were found beyond the tubular basement membrane (Figure 1D). CD44 staining was also present in Bowman's capsule of secondarily damaged glomeruli in *kdkd* kidney. In normal and in *kdkd* mice there was also positive CD44 staining in the basal layer of the papillary epithelium (not shown).

We then examined *kdkd* kidney RNA for CD44 mRNA transcripts. Figure 2 shows the RT-PCR analysis for CD44 gene expression, using primers that flank the variant insertion site between exons 5s and 6s [16]. A weak 221 bp CD44 fragment reflecting standard CD44 was visible in young *kdkd* kidney by Southern blot analysis. The level of this fragment was enhanced several times as the disease progressed, but remained at low levels in normal control mice (not shown). Furthermore, additional transcripts (3 to 4) encoding variant forms of CD44 were detected in older and more severely diseased *kdkd* mice.

Increased hyaluronan staining in kdkd *kidney and colocalization with CD44*

We then examined normal and *kdkd* kidneys for the presence of HA, the major ligand for CD44. Figure 3 demonstrates that almost no HA staining was visible in the cortex in normal CBA/CaH mice, except around a few glomeruli (Figure 3A). HA was present weakly in the renal interstitium of the medulla in normal

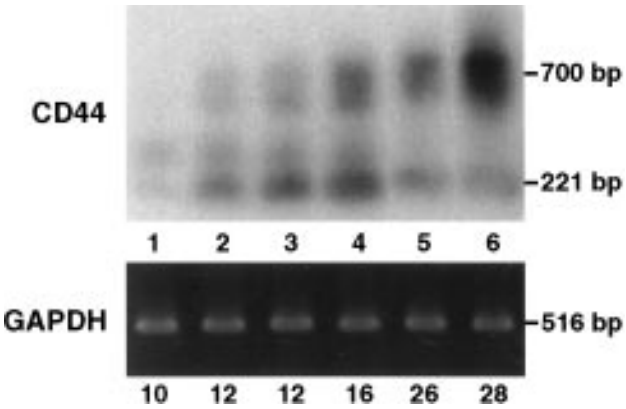

Fig. 2. RT-PCR and Southern blot analysis of CD44 mRNA expression in *kdkd* kidney, using CD44 primers that flank the variant exon insertion site. The lanes reflect mRNA from individual mice. Increased levels of the standard 221 bp fragment are detected in *kdkd* mice. Additional variant mRNA forms of CD44 are present in increased amounts in *kdkd* kidney. Numbers at the bottom reflect age of mice in weeks.

CBA/CaH mice (Figure 3C). HA was also present in the adventitia of large blood vessels (not shown). In *kdkd* kidneys, however, there was a marked increase in HA staining both in the cortex (Figure 3B) and in the medulla (Figure 3D). Incubation of sections with hyaluronidase eliminated fluorescence staining completely, proving the specificity of the HA staining (not shown). The *de novo* appearance of HA in the cortical interstitium was mainly seen around CD44-positive interstitial cells and around damaged, collapsed, and dilated CD44-positive tubules. CD44 expression did not precede HA deposition, both occurred in parallel. Figure 4 shows that HA staining was most abundant in the interstitial space adjacent to tubules which display strong CD44 staining basolaterally.

Augmented osteopontin expression correlates with renal disease in kdkd *mice*

It has recently been shown that CD44 is a receptor not only for HA but also for the chemotactic protein Opn [20]. By virtue of an RGD sequence Opn is also known to bind to the vitronectin receptor $\alpha_v\beta_3$ and to

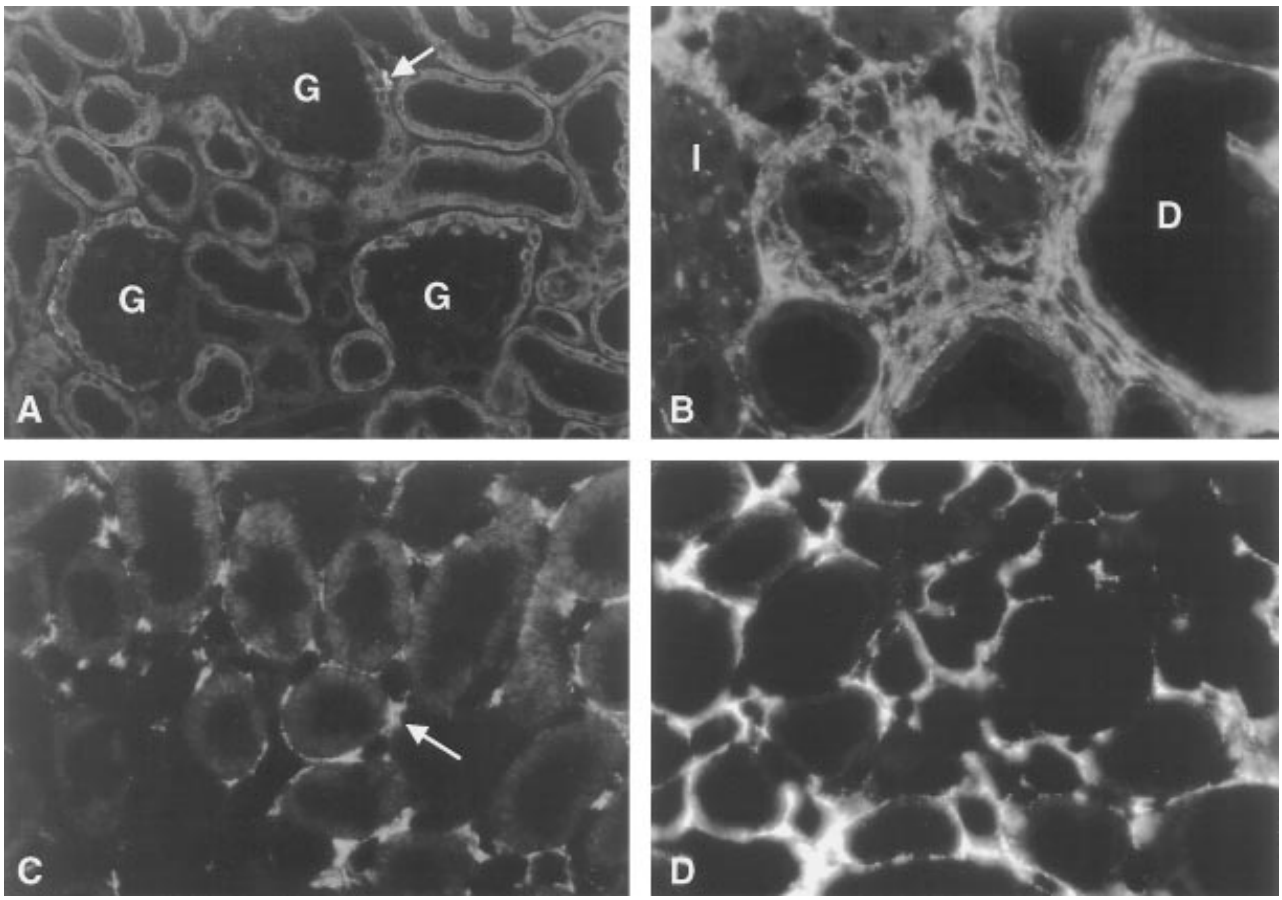

Fig. 3. Immunofluorescence staining for hyaluronan (HA) in 4-month-old CBA/CaH normal control and *kdkd* mouse. **A**, CBA/CaH mouse, cortex. The cortical interstitium has virtually no HA staining, except around a few glomeruli (G) (arrow) (magnification ×400). **B**, *Kdkd* mouse. The interstitium around injured (I) and dilated (D) cortical tubules and around infiltrating cells stains intensely for HA (×1000). **C**, CBA/CaH mouse, medulla. Weak HA staining is seen around TAL (arrow) (×1000). **D**, *Kdkd* mouse, medulla. Strong HA staining in a confluent reticular network pattern is detected between tubules (×1000).

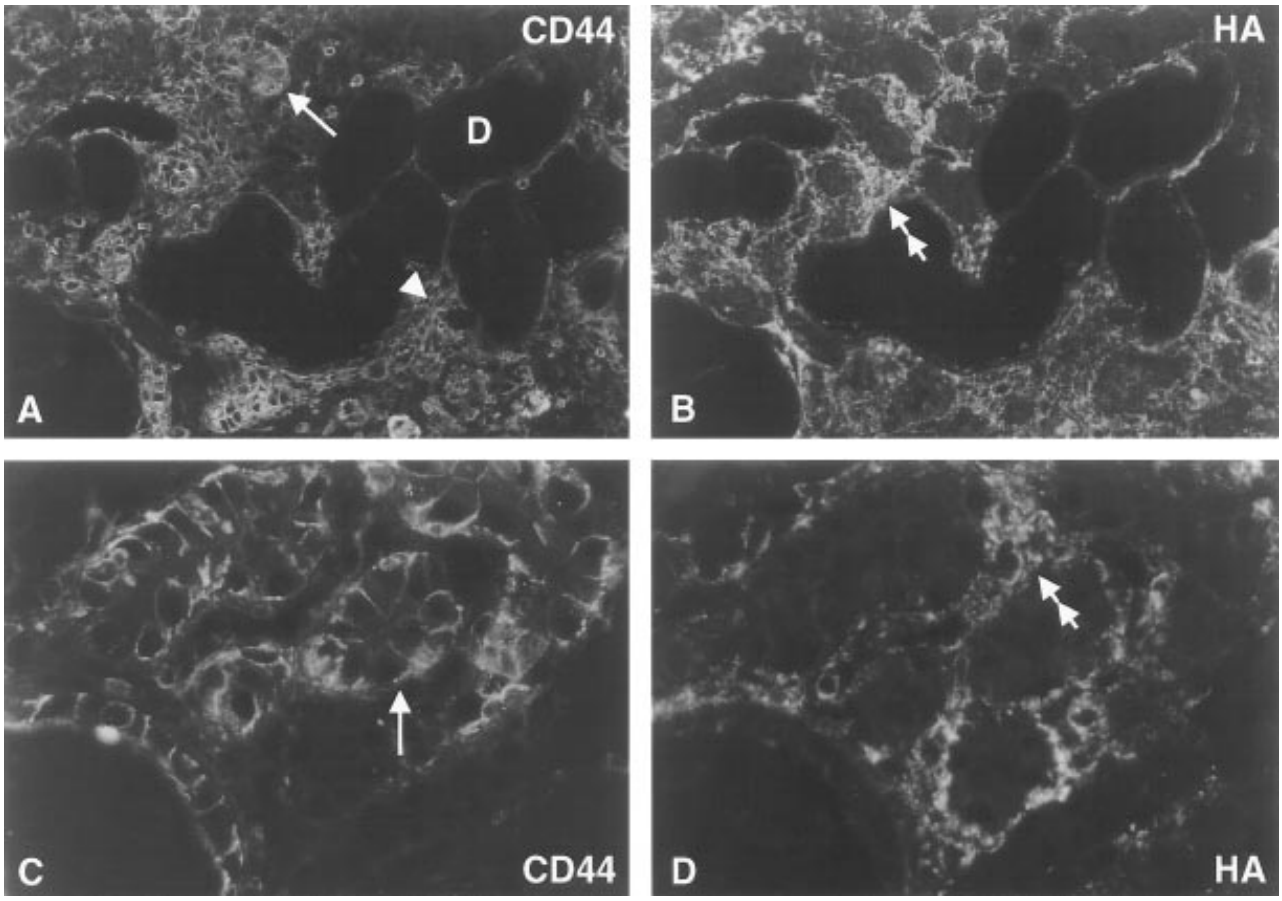

Fig. 4. Colocalization of CD44 (**A**,**C**) and HA (**B**,**D**) in 4-month-old *kdkd* mouse. Sections were double-stained for CD44 and HA, using anti-CD44 mAb and b-PG, and FITC- or Cy3-labelled secondary reagents respectively. Sections were photographed using separate filters for CD44 and Opn. **A** and **C**, CD44 is strongly expressed by injured (arrow) and dilated tubules (D) and by infiltrating cells (arrowhead). **B** and **D**, Abundant HA surrounds injured tubules (double arrow). Note the close association of CD44 with HA (compare **A** with **B** and **C** with **D**) which suggests interaction of CD44 with its ligand HA *in vivo*. Magnification × 250 (**A** and **B**) and × 1000 (**C** and **D**).

the integrins $\alpha_v\beta_1$ and $\alpha 12_v\beta_5$ [21]. Because the interaction of Opn with upregulated epithelial CD44 could be important in the pathogenesis of *kdkd* nephritis we sought to correlate CD44 expression in *kdkd* TIN with the expression of Opn by immunofluorescence staining and Northern blot analysis. Figure 5 shows the immunofluorescence staining results for Opn in normal and *kdkd* kidney. Constitutive expression of Opn could be detected in distal tubules (thick ascending limb (TAL), distal convoluted tubule (DCT) and connecting tubule (CNT)) and in the papillary epithelium in normal kidneys (Figure 5A and 5B). The expression of Opn was markedly enhanced at these sites in *kdkd* kidneys. Furthermore, Opn staining also appeared in proximal tubules, particularly in areas of tubular injury (Figure 5C and 5D). The staining pattern in proximal tubules was granular and cytoplasmic with a perinuclear accentuation and also subapical. Figure 6 shows the Northern blot analysis of Opn expression in normal and *kdkd* kidneys. In agreement with the immunofluorescence data, constitutive Opn expression was found in normal kidneys. *Kdkd* displayed a 2–5-fold increase in the steady-state Opn transcript levels, whereas levels for the housekeeping gene GAPDH varied little, suggesting specific upregulation of Opn mRNA transcripts in *kdkd* nephritis. Opn levels were highest in the most severely injured kidneys.

Colocalization of CD44 and Opn

Since Opn is known to be a ligand for CD44, we then sought to colocalize CD44 and Opn staining in *kdkd* kidneys (Figure 7). In *kdkd* mice injured proximal tubules displayed both CD44 and Opn staining. The CD44 staining pattern was mainly located at the cell surface with some basolateral accentuation, whereas the Opn staining was cytoplasmic with apical accentuation or in the perinuclear region where the Golgi apparatus is situated. Colocalization was most prominent on severely injured and collapsed tubules (Figure 7A and 7B), and on tubules with altered epithelial cell morphology (Figure 7C and 7D). CD44 did not precede Opn expression on injured tubules; both appeared in parallel and at the same time. We also observed that the papillary epithelium was CD44 positive in its basal layer, whereas the apical cells expressed mainly Opn but no CD44 (not shown).

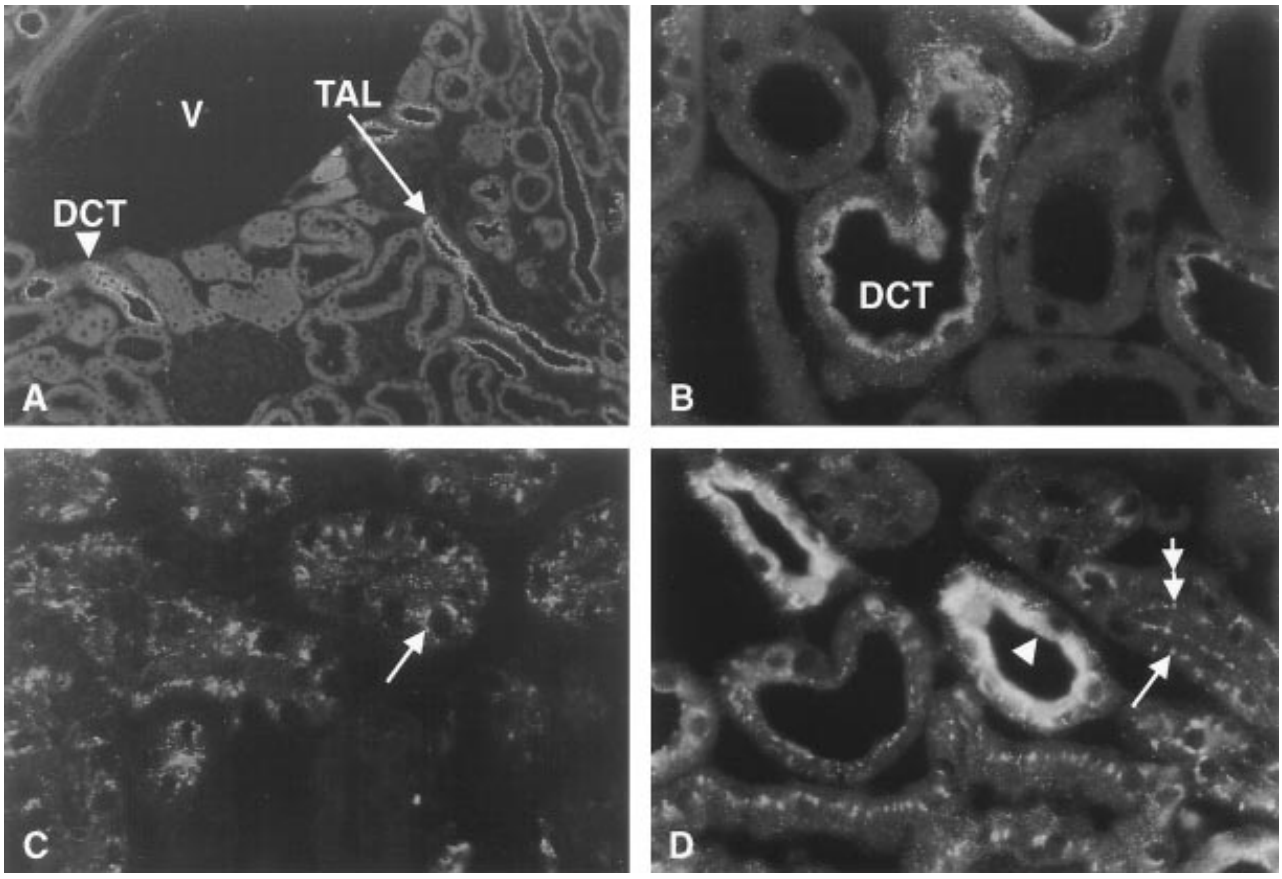

Fig. 5. Immunofluorescence staining for Opn in 4-month-old normal control CBA/CaH and *kdkd* mice. **A**, CBA/CaH mouse. Constitutive fine-granular apical staining is present in TAL (arrow) and DCT (arrowhead) close to a vein (V). Note absent proximal tubular staining (magnification ×250). **B**, CBA/CaH mouse. Positive Opn staining on DCT (×1000). **C**, *Kdkd* mouse. Collapsed tubules (arrow) express Opn abundantly (×1000). **D**, *Kdkd* mouse. DCT have markedly increased Opn expression apically (arrowhead) compared with CBA/CaH (**B**). Surrounding proximal tubules show specific fluorescence in the perinuclear region (arrows) and apically under the brush border in a fine-granular pattern (double arrow) (×1000).

Discussion

CD44 is a widely distributed matrix receptor present on haematopoietic and non-haematopoietic cells [22]. CD44 is known to regulate cell adhesion and cell movement through interaction with the matrix constituent hyaluronic acid (HA) and—as recently demonstrated [20]—with the phosphoprotein osteopontin (Opn). Various studies have documented enhanced expression of CD44 in inflammatory lesions, for example in human rheumatoid arthritis [23] and in collagen-induced arthritis in mice [24]. Several groups including our own have reported a striking induction of CD44 in glomeruli and also in tubules in various renal disease states. Enhanced CD44 expression has thus been demonstrated in murine SLE [7], anti-Thy-1 nephritis in the rat [8,25], and also in human kidney with SLE and IgA nephritis [8].

The striking feature in our previous study in MRL-*Fas*^{lpr} mice with lupus nephritis was the induction of CD44 in cortical tubular epithelial cells [7]. We have speculated that the *de novo* expression of epithelial CD44 (perhaps induced by proinflammatory cytokines) could participate in the autoimmune interstitial nephritic process in this model. We were thus particularly interested to examine CD44 expression in renal diseases where the tubular lesion is the primary event, and have therefore examined *kdkd* mice as a genetically determined model of TIN. We show here that CD44 is markedly induced on injured cortical TEC in *kdkd* mice but is absent in congenic CBA/CaH mice. We also report that CD44 colocalizes with its major ligand HA in areas of nephron injury, suggesting *in vivo* interaction between HA and its receptor in this model of TIN.

HA is a high-molecular-weight glycosaminoglycan found normally in the extracellular matrix, particularly in soft connective tissues. HA is synthesized by mesenchymal cells and fibroblasts and stabilizes tissue architecture [26]. HA has a limited distribution in the kidney and is normally present only in the medullary interstitium, where it may play a role in the mechanical stability of the tubules in this region of the kidney [27]. It has been shown that HA synthesis is increased in inflammatory renal diseases such as in human and rat renal allograft rejection [28–30] and anti-GBM

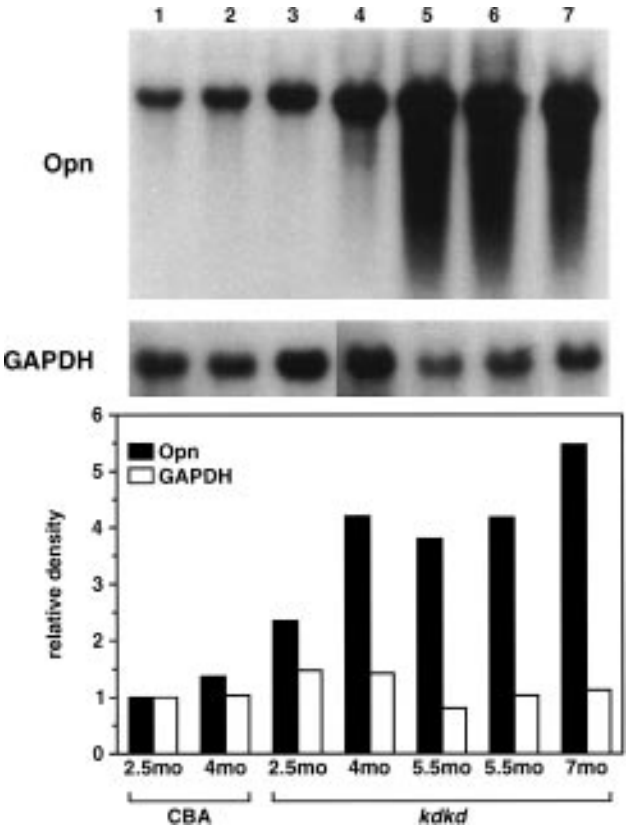

Fig. 6. Northern blot analysis for Opn and GAPDH gene expression in the kidney of normal congenic CBA/CaH and *kdkd* mice. Densitometric analysis is shown in the lower part of the Figure. Each lane represents an individual animal. Lanes 1, 2 demonstrate constitutive mRNA expression for Opn in two normal mice. Lanes 3–7 show enhanced Opn mRNA levels in *kdkd* kidney.

nephritis in the rat [31], particularly in areas of immune injury. As HA has a high water-binding capacity it has been speculated that it could be responsible for tissue oedema formation in the rejecting kidney [30].

Here we show for the first time that *kdkd* mice also display a striking increase in interstitial HA content, particularly in the cortex in areas of mononuclear cell infiltration and tubular injury. HA staining correlates well with CD44 expression, suggesting *in vivo* interaction between HA and its major receptor CD44. What could be the functional significance of HA accumulation in the renal cortex of *kdkd* mice? Abundant HA could facilitate the migration of CD44-positive leukocytes into areas of tubular inflammation, in analogy with the promotion of locomotion of *ras*-transformed fibroblasts [32]. HA is also known to influence macrophage function critically. Depending on its concentration, HA has stimulatory or inhibitory effects on phagocytosis. Concentrated, viscous HA generally has inhibitory effects, whereas dilute HA may stimulate phagocytosis and other cell activities (reviewed in [26]). Furthermore, the interaction of HA with CD44 on macrophages has been shown to stimulate the release of proinflammatory cytokines such as TNF-α [33,34]. There is also evidence that low-molecular-weight fragments of HA but not the high-molecular forms cause macrophage activation through NF-κB [35]. It remains to be determined whether these HA fragments are also found *in vivo*. It is tempting to speculate, however, that the interstitial accumulation of HA and its low-molecular forms in the renal cortex could modulate the activation and function of infiltrating macrophages in *kdkd* mice. Promotion of phagocytosis and TNF-α release could be important mechanisms in the disease process in *kdkd* mice.

The biosynthetic pathways and the degradation mechanisms of renal HA have not been investigated. Presently we do not know which cells produce HA in the cortex of *kdkd* mice. It has been shown that CD44 participates in the uptake and degradation of hyaluronan by fibroblasts and macrophages [36,37], suggesting that CD44 plays a role in hyaluronan removal and metabolism. We speculate that in areas of injury, regenerating tubules could regulate HA content though their CD44 receptor. Further studies will be required to investigate whether tubular cells expressing CD44 play a role in HA metabolism *in vivo*.

It has recently been demonstrated that CD44 also functions as a receptor for the phosphoprotein Opn [20]. Opn interaction with CD44 induces cellular chemotaxis, whereas the interaction of HA with CD44 promotes cell adhesion. Thus, depending on its ligand, CD44 may trigger differing cellular functions [20]. Opn was originally isolated from rat bone but was subsequently cloned from other sources, including activated T cells (reviewed in [38]). It has also been termed uropontin and Eta-1 (for **E**arly **T** lymphocyte **a**ctivation 1). Its expression in the normal kidney has been examined in various species, including mouse, rat, and human [14,39,40]. Opn has diverse physiological functions and is important in bone development and remodelling. More important for our discussion, Opn can also function as a chemotactic protein and has been shown to induce a macrophage-rich infiltrate when injected subcutaneously in mice [41]. Several acute rat renal disease models have been examined for Opn expression. Thus, Opn is increased in rat anti-Thy-1 nephritis, puromycin nephrosis, and passive Heymann nephritis [42], as well as in angiotensin-II-induced TIN [43], protein-overload proteinuria [44], and cyclosporin A nephrotoxicity [45]. In all these models Opn expression is also increased in proximal tubules and generally correlates with the presence of macrophages in the interstitium.

In agreement with other studies we also found that Opn expression in normal mice is confined to distal tubules, namely the TAL of Henle's loop, the DCT and the CNT, and also to the papillary epithelium [39]. Our results show that Opn is markedly overexpressed in *kdkd* mice, a chronic model of TIN. Consistent with the expression pattern in the above-mentioned acute renal disease models, Opn staining extends to the proximal tubules in *kdkd* mice where the macrophage infiltration is prominent, suggesting that Opn could participate in the recruitment of mono-

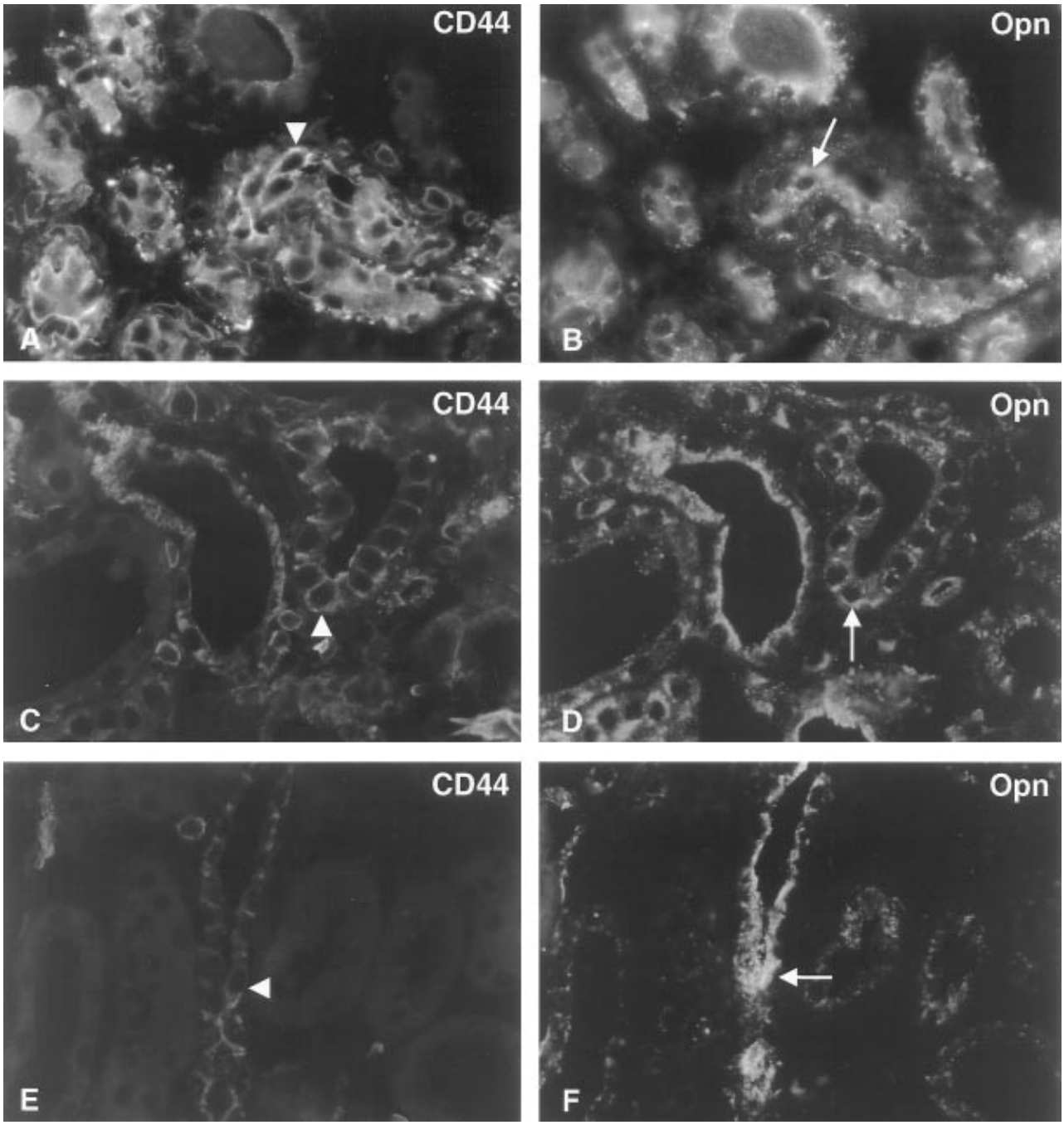

Fig. 7. Colocalization of CD44 (**A**,**C**,**E**) and Opn (**B**,**D**,**E**) in *kdkd* mice. **A**,**B**, Severely injured tubule (arrowhead in **A**) displays strong CD44 staining that colocalizes with Opn (arrow in **B**). **C**,**D**, CD44 (arrowhead in **C**) also colocalizes with Opn (arrow in **D**) in less severely injured and regenerating TEC. **E**,**F**, CD44 also colocalizes with Opn in TAL. Staining patterns are accentuated on the cell surface for CD44 (arrowhead in **E**), and cytoplasmic/apical for Opn (arrow in **F**) (magnifications $\times 1000$).

nuclear phagocytic cells in *kdkd* nephritis. Interestingly, Opn mRNA transcript levels are chronically upregulated in *kdkd* kidney compared with age-matched congenic CBA/CaH mice, and levels correlate with the severity of the renal disease.

Opn colocalizes with CD44 in areas of tubular lesions in *kdkd* kidneys. Autocrine secretion of Opn could regulate TEC function through CD44, since CD44 has known signalling capabilities [22]. Because of its apical location Opn could also be secreted in the proximal tubular lumen and influence immune events in distal nephron segments to participate in the recruitment of monocytes/macrophages. In *kdkd* mice macrophages are present abundantly and we suspect that Opn could play an important role in macrophage influx in *kdkd* nephritis.

In summary, we have shown that CD44 is expressed *de novo* by tubular epithelial cells in areas of cortical

tubular injury in the *kdkd* model of autoimmune TIN, but not in normal control kidneys. CD44 and HA staining patterns suggest that tubular CD44 binds accumulated HA in the interstitium. The precise function of this interaction remains to be determined. The overexpression of Opn by injured proximal tubular cells is prominent in *kdkd* mice. The interaction of CD44 with this chemoattractant might be of pathophysiological importance and could provide a CD44-mediated signal for TEC and for macrophages, altering tubular function and promoting macrophage influx into the kidney of *kdkd* mice.

Acknowledgements. Part of this study was presented at the 2nd meeting of the European Kidney Research Forum in May 1996 in Baveno, Italy and has been published in abstract form (*Kidney Int* 1996; 50: 1787A). This study was supported by the Swiss National Science Foundation (grant No. 32–40390.94 to RPW), the Olga–Mayenfisch Foundation and the Hartmann–Müller Foundation. RPW is the recipient of a SCORE A Physician Scientist Award (grant No. 32–38821.93) from the Swiss National Science Foundation. XF is the recipient of a Visitor Scholarship from the Swiss government. We gratefully acknowledge the gift of b-PG by Dr Charles Underhill, and also thank Dr Elaine Worcester for the Opn antiserum.

References

1. Lyon MF, Hulse EV. An inherited kidney disease of mice resembling human nephronophthisis. *J Med Gen* 1971; 8: 41–48
2. Smoyer WE, Kelly CJ. Inherited interstitial nephritis in *kdkd* mice. *Int Rev Immunol* 1994; 11: 245–251
3. Neilson EG, McCafferty E, Feldman A, Clayman MD, Zakheim B, Korngold R. Spontaneous interstitial nephritis in *kdkd* mice. I. An experimental model of autoimmune renal disease. *J Immunol* 1984; 133: 2560–2565
4. Kelly CJ, Neilson EG. Contrasuppression in autoimmunity. Abnormal contrasuppression facilitates expression of nephritogenic effector T cells and interstitial nephritis in *kdkd* mice. *J Exp Med* 1987; 165: 107–123
5. Kelly CJ, Korngold R, Mann R, Clayman M, Haverty T, Neilson EG. Spontaneous interstitial nephritis in *kdkd* mice. II. Characterisation of a tubular antigen-specific, H-2^K-restricted Lyt-2^+ effector T cell that mediates destructive tubulointerstitial injury. *J Immunol* 1986; 136: 526–531
6. Neilson EG. The nephritogenic T lymphocyte response in interstitial nephritis. *Semin Nephrol* 1993; 13: 496–502
7. Benz PS, Fan XH, Wüthrich RP. Enhanced tubular epithelial CD44 expression in MRL-*lpr* lupus nephritis. *Kidney Int* 1996; 50: 156–163
8. Roy-Chaudhury P, Khong TF, Williams JH *et al.* CD44 in glomerulonephritis: expression in human renal biopsies, the Thy 1.1 model, and by cultured mesangial cells. *Kidney Int* 1996; 50: 272–281
9. Trowbridge IS, Lesley J, Schulte R, Hyman R, Trotter J. Biochemical characterisation and cellular distribution of a polymorphic, murine cell-surface glycoprotein expressed on lymphoid tissues. *Immunogenetics* 1982; 15: 299–312
10. Miyake K, Medina KL, Hayashi S, Ono S, Hamaoka T, Kincade PW. Monoclonal antibodies to Pgp-1/CD44 block lympho-hemopoiesis in long-term bone marrow cultures. *J Exp Med* 1990; 171: 477–488
11. Lesley J, He Q, Miyake K, Hamann A, Hyman R, Kincade PW. Requirements for hyaluronic acid binding by CD44: a role for the cytoplasmic domain and activation by antibody. *J Exp Med* 1992; 175: 257–266
12. Green SJ, Tarone G, Underhill CB. Distribution of hyaluronate and hyaluronate receptors in the adult lung. *J Cell Sci* 1988; 90: 145–156
13. Worcester EM, Blumenthal SS, Beshensky AM, Lewand DL. The calcium oxalate crystal growth inhibitor protein produced by mouse kidney cortical cells in culture is osteopontin. *J Bone Miner Res* 1992; 7: 1029–1036
14. Kleinman JG, Beshensky A, Worcester EM, Brown D. Expression of osteopontin, a urinary inhibitor of stone mineral crystal growth, in rat kidney. *Kidney Int* 1995; 47: 1585–1596
15. Chirgwin JM, Przybyla AE, MacDonald RJ, Rutter WJ. Isolation of biologically active ribonucleic acid from sources enriched in ribonuclease. *Biochemistry* 1979; 18: 5294–5299
16. Hirano H, Screaton GR, Bell MV, Jackson DG, Bell JI, Hodes RJ. CD44 isoform expression mediated by alternative splicing: tissue-specific regulation in mice. *Int Immunol* 1994; 6: 49–59
17. Zhou DFH, Ding JF, Picker LJ, Bargatze RF, Butcher EC, Goeddel DV. Molecular cloning and expression of Pgp-1. The mouse homolog of the human H-CAM (Hermes) lymphocyte homing receptor. *J Immunol* 1989; 143: 3390–3395
18. Feinberg AP, Vogelstein B. A technique for radiolabeling DNA restriction endonuclease fragments to high specific activity. *Anal Biochem* 1983; 132: 6–13
19. Craig AM, Smith JH, Denhardt DT. Osteopontin, a transformation-associated cell adhesion phosphoprotein, is induced by 12-O-tetradecanoylphorbol 13-acetate in mouse epidermis. *J Biol Chem* 1989; 264: 9682–9689
20. Weber GF, Ashkar S, Glimcher MJ, Cantor H. Receptor-ligand interaction between CD44 and osteopontin (Eta-1). *Science* 1996; 271: 509–512
21. Hu DD, Lin EC, Kovach NL, Hoyer JR, Smith JW. A biochemical characterization of the binding of osteopontin to integrins $\alpha_v\beta_1$ and $\alpha_v\beta_5$. *J Biol Chem* 1995; 270: 26232–26238
22. Lesley J, Hyman R, Kincade PW. CD44 and its interaction with extracellular matrix. *Adv in Immunol* 1993; 54: 271–335
23. Haynes BF, Hale LP, Patton KL, Martin ME, McCallum RM. Measurement of an adhesion molecule as an indicator of inflammatory disease activity. Up-regulation of the receptor for hyaluronate (CD44) in rheumatoid arthritis. *Arthritis Rheum* 1991; 34: 1434–1443
24. Mikecz K, Brennan FR, Kim JH, Glant TT. Anti-CD44 treatment abrogates tissue oedema and leukocyte infiltration in murine arthritis. *Nature Medicine* 1995; 1: 558–563
25. Nikolic-Paterson DJ, Jun Z, Tesch GH, Lan HY, Foti R, Atkins RC. De novo CD44 expression by proliferating mesangial cells in rat anti-Thy-1 nephritis. *J Am Soc Nephrol* 1996; 7: 1006–1014
26. Laurent TC, Fraser JRE. Hyaluronan. *FASEB J* 1992; 6: 2397–2404
27. Pitcock JA, Lyons H, Brown PS, Rightsel WA, Muirhead EE. Glycosaminoglycans of the rat renomedullary interstitium: ultrastructural and biochemical observations. *Exp Mol Pathol* 1988; 49: 373–387
28. Wells AF, Larsson E, Tengblad A *et al.* The localization of hyaluronan in normal and rejected human kidneys. *Transplantation* 1990; 50: 240–243
29. Wells A, Larsson E, Hanas E, Laurent T, Hällgren R, Tufveson G. Increased hyaluronan in acutely rejecting human kidney grafts. *Transplantation* 1993; 55: 1346–1349
30. Hällgren R, Gerdin B, Tufveson G. Hyaluronic acid accumulation and redistribution in rejecting rat kidney graft. Relationship to the transplantation edema. *J Exp Med* 1990; 171: 2063–2076
31. Nishikawa K, Andres G, Bhan AK *et al.* Hyaluronate is a component of crescents in rat autoimmune glomerulonephritis. *Lab Invest* 1993; 68: 146–153
32. Turley EA, Austen L, Vandeligt K, Clary C. Hyaluronan and a cell-associated hyaluronan binding protein regulate the locomotion of *ras*-transformed cells. *J Cell Biol* 1991; 112: 1041–1047
33. Noble PW, Lake FR, Henson PM, Riches DW. Hyaluronate activation of CD44 induces insulin-like growth factor-1 expression by a tumor necrosis factor-α-dependent mechanism in murine macrophages. *J Clin Invest* 1993; 91: 2368–2377
34. Lake FR, Noble PW, Henson PM, Riches DW. Functional switching of macrophage responses to tumor necrosis factor-α (TNF-α) by interferons. Implications for the pleiotropic activities of TNF-α. *J Clin Invest* 1994; 93: 1661–1669
35. Noble PW, McKee CM, Cowman M, Shin HS. Hyaluronan

fragments activate an NFκB/I-κBα autoregulatory loop in murine macrophages. *J Exp Med* 1996; 183: 2373–2378
36. Culty M, Nguyen HA, Underhill CB. The hyaluronan receptor (CD44) participates in the uptake and degradation of hyaluronan. *J Cell Biol* 1992; 116: 1055–1062
37. Underhill CB, Nguyen HA, Shizari M, Culty M. CD44 positive macrophages take up hyaluronan during lung development. *Dev Biol* 1993; 155: 324–336
38. Denhardt DT, Guo X. Osteopontin: a protein with diverse functions. *FASEB J* 1993; 7: 1475–1482
39. Lopez CA, Hoyer JR, Wilson PD, Waterhouse P, Denhardt DT. Heterogeneity of osteopontin expression among nephrons in mouse kidneys and enhanced expression in sclerotic glomeruli. *Lab Invest* 1993; 69: 355–363
40. Brown LF, Berse B, van de Water L *et al.* Expression and distribution of osteopontin in human tissues: widespread association with luminal epithelial surfaces. *Mol Biol Cell* 1992; 3: 1169–1180
41. Singh RP, Patarca R, Schwartz J, Singh P, Cantor H. Definition of a specific interaction between the early T lymphocyte activation 1 (Eta-1) protein and murine macrophages *in vitro* and its effects upon macrophages *in vivo*. *J Exp Med* 1990; 171: 1931–1942
42. Pichler R, Giachelli CM, Lombardi D *et al.* Tubulointerstitial disease in glomerulonephritis. Potential role of osteopontin (uropontin). *Am J Pathol* 1994; 144: 915–926
43. Giachelli CM, Pichler R, Lombardi D *et al.* Osteopontin expression in angiotensin II-induced tubulointerstitial nephritis. *Kidney Int* 1994; 45: 515–524
44. Eddy AA, Giachelli CM. Renal expression of genes that promote interstitial inflammation and fibrosis in rats with protein-overload proteinuria. *Kidney Int* 1995; 47: 1546–1557
45. Young BA, Burdmann EA, Johnson RJ *et al.* Cellular proliferation and macrophage influx precede interstitial fibrosis in cyclosporine nephrotoxicity. *Kidney Int* 1995; 48: 439–448

Received for publication: 28.11.96
Accepted in revised form: 11.3.97