CD44 Disruption Prevents Degeneration of the Capillary Network in Obstructive Nephropathy *via* Reduction of TGF-β1–Induced Apoptosis

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CD44 is a glycoprotein that is involved in inflammation and cell–cell/cell–matrix interactions, is upregulated in the kidney upon injury, and leads to fibrosis through enhancement of TGF-β1 signaling. Absence of CD44 prevents development of renal fibrosis in unilateral ureteral obstruction (UUO). A hallmark of development of renal fibrosis is the degeneration of the capillary network. This study shows that CD44 is upregulated on capillary endothelial cells during UUO. For elucidation of the role of CD44 on peritubular endothelial cells in UUO, capillary network degeneration was compared in $CD44^{+/+}$ and $CD44^{-/-}$ mice. As expected, degeneration of the capillary network was observed in $CD44^{+/+}$ mice during UUO, associated with increased endothelial apoptosis. However, in the absence of CD44, degeneration of the network is prevented as a result of a decrease in the rate of apoptosis in endothelial cells. The divergence in endothelial apoptosis is not correlated to differential vascular endothelial growth factor or thrombospondin-1 expression. For further investigation of capillary regression, $CD44^{+/+}$ and $CD44^{-/-}$ peritubular capillary endothelial cell lines were established. With the use of these cells, it is shown that interaction between CD44 and its ligand hyaluronic acid enhances the proapoptotic effect of TGF-β1 but not thrombospondin-1 on endothelial cells, contributing to the degeneration of the capillary network. Blocking interaction between hyaluronic acid and CD44 therefore may be a potential therapeutic opportunity to preserve the capillary network and prevent the development of fibrosis in chronic renal disease.

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An important determinant of progression of renal disease is tubulointerstitial damage, histologically characterized by tubular dilation, atrophy, and accumulation of extracellular matrix (ECM) proteins that lead to the development of fibrosis. CD44 is a family of glycoproteins that are encoded by a single gene that consists of 19 exons. Alternative splicing generates different isoforms and confers specific functions to the CD44 protein (1–4). These isoforms have been implicated in many important physiologic and pathologic processes, such as cell–cell and cell–matrix interaction, lymphocyte extravasation, wound healing/scarring, cell migration, lymphocyte activation, and binding/presentation of growth factors (5–8).

The shortest (standard) isoform of CD44 does not contain any of the alternative domains and binds hyaluronic acid (HA) and osteopontin, which are two major components of the extracellular matrix. The interaction between CD44 and HA is involved in extravasation of inflammatory cells (9,10) and facilitates TGF-β1 signaling (11,12). CD44 isoforms that contain the domain encoded by variable exon 3 (CD44v3) contain a heparan sulfate–binding site and therefore are able to bind several growth factors and facilitate their signaling (8).

CD44 is not expressed in the normal kidney, but it is rapidly upregulated after injury in a number of animal models for kidney diseases (10,12–15) and human nephropathies (16,17). Previously, we showed that CD44 is crucial in the development of renal fibrosis through enhancement of TGF-β1 signaling (12). CD44 is also involved in the maintenance of tubular architecture and reduces tubular damage in unilateral ureteral obstruction (UUO) by enhancement of beneficial growth factor signaling, such as hepatocyte growth factor (12,18).

An important contributor to persistent renal injury and progressive interstitial fibrosis is the loss of peritubular capillaries (19). Endothelial cell loss is associated with both increased cell death and decreased cell proliferation. Accordingly, preservation of the capillary network prevented development of fibrosis in the remnant kidney (20–22).

Various studies have underlined the opposing functions of vascular endothelial growth factor (VEGF) and thrombospondin-1 (TSP-1) in the process of capillary degeneration. In normal kidney, the angiogenic, survival, and trophic factor VEGF is constitutively expressed mainly on tubular epithelial cells. In progressive renal disease, the expression of VEGF is altered (20–22). In contrast, TSP-1 exerts powerful antiangiogenic actions by counteracting VEGF-mediated endothelial cell proliferation (23), and it induces endothelial cell apoptosis (24–26). Expression of TSP-1 is restricted mainly to the interstitium

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of damaged kidneys. Because both VEGF and TSP-1, the two major mediators implicated in capillary network preservation/regression, are heparan sulfate–binding proteins (24,27) and thus theoretically able to bind CD44v3, leading to enhanced signaling, we examined the role of CD44 in the degeneration of the peritubular capillary endothelial network during UUO.

Materials and Methods

Mice and Experimental Protocol

CD44 knockout on C57Bl/6 background ($CD44^{-/-}$) (28) and C57Bl/6 wild type ($CD44^{+/+}$) male mice (6 to 8 wk old) were bred in our animal facility. Right kidney UUO or sham surgery was performed as described previously (12). Mice were killed humanely ($n = 6$ for each group) at days 1, 3, 7, and 14 after UUO or sham surgery. All experimental procedures were approved by the Animal Care and Use Committee of the University of Amsterdam (Amsterdam, The Netherlands).

Histology and Immunohistochemistry

Renal tissues were fixed in 10% formalin for 12 h and embedded in paraffin in a routine manner. For immunohistochemistry, antigen retrieval was performed by incubation in a 10-mM sodium citrate solution (pH 6.0) for 10 min at 98°C in a microwave oven, except for VEGF (clone ab-4; Calbiochem, Darmstadt, Germany) and TSP-1 (Calbiochem), which was performed in 10 mM Tris/1 mM EDTA (pH 9). After endogenous peroxidase activity was blocked with 0.3% H_2O_2 in methanol and free protein binding sites were blocked with normal goat serum, sections were probed with the antibody. As a negative control, we used species- and isotype-matched antibodies. After incubation with the secondary (horseradish peroxidase [HRP] labeled) antibodies (DAKO, Glostrup, Denmark), bound antibodies were visualized by developing peroxidase activity using 3,3-diamino-benzidine tetrachloride (Sigma Chemical Co., St. Louis, MO). Double immunostaining for endothelial cells and CD44, proliferation, or apoptosis was identified by immunostaining with CD34 (Cedarlane, Hornby, ON, Canada) and respectively anti-CD44 (clone IM7.8.1; ATCC, Livermore, CA), anti–proliferating cell nuclear antigen (anti-PCNA; DAKO), or anti-active caspase-3 (Cell signaling Technology, Beverly, MA). The anti-CD34 antibody was probed with a secondary antibody anti-rat IgG2a–alkaline phosphatase (AP) (Zymed, San Francisco, CA), CD44 with anti-rat IgG2b-HRP, PCNA with an anti-mouse IgG2a-HRP, and caspase-3 with anti-rabbit IgG-HRP. AP activity was visualized with Vector Blue, and HRP activity was visualized with Novared (Vector Laboratories, Burlingame, CA).

Flow Cytometric Analysis

Before surgery, 3 and 7 d after UUO, kidneys were harvested and single-cell suspensions were made by straining the tissue through a 40-μm mash. Erythrocytes were subjected to hypotonic lysis. The cell suspensions were incubated with anti–CD34-FITC and CD44-PE (both BD Pharmingen, Erembodegem, Belgium) for 60 min at 4°C. CD44 expression on endothelial cells was analyzed by flow cytometry (FACSCalibur, Becton Dickinson, Franklin Lakes, NJ).

Histopathologic Scoring

CD34-positive areas and TSP-1 expression in the renal cortex were analyzed using a digital image analysis program (Image pro-plus, Mediacybernetics, Gleichen, Germany); values are expressed as a percentage of the total cortex. Glomeruli and arteries were excluded from quantification. To evaluate the number of proliferating and apoptotic peritubular capillary endothelial cells (CD34-positive cells), we analyzed 10 randomly chosen, nonoverlapping fields (×200). Values are expressed as cells/mm^2. VEGF expression was analyzed as the percentage VEGF-expressing tubuli.

Isolation of Peritubular Capillary Endothelial Cells

Immortomice (CBA/ca × C57Bl/10 hybrid; Charles River Laboratories, Maastricht, The Netherlands) were crossed with $CD44^{+/+}$ and $CD44^{-/-}$ for more than four generations. Kidneys of $CD44^{+/+}$ and $CD44^{-/-}$ mice homozygous for the temperature-sensitive SV40 large T antigen (H-2k^b-tsA58) were collected. Removal of the glomeruli was performed as described by Takemoto *et al.* (29). In short, kidneys were perfused with 10×10^7 magnetic beads (Ø 4.5 μm; Sigma) in PBS. Kidneys were cut into small pieces (approximately 1 mm^3) and digested in 1 mg/ml collagenase A (Roche Diagnostics, Mannheim, Germany) in Hanks balanced salt solution, for 15 min at 37°C. The digested kidneys were pressed through a 100-μm cell strainer. Glomeruli were removed using a magnetic particle concentrator. In addition, the remaining cell suspension was filtered through a 40-μm cell strainer to remove any residual glomeruli.

Peritubular capillary endothelial cells were isolated as described by Langley *et al.* (30). In short, the remaining cell suspension was grown in DMEM supplemented with 10% FCS and 2 mM L-glutamine (all Life Technologies, Rockville, MD) and 25 ng/ml IFN-γ (R&D Systems, Minneapolis, MN) at 33°C until confluence. Confluent cells then were placed for 8 d at 37°C without addition of IFN-γ. These cells then were stimulated using 10 ng/ml murine TNF-α (R&D Systems) for 4 h. Cells were labeled with vascular cellular adhesion molecule-1 (VCAM-1)–FITC and E-selectin–PE (both BD Pharmingen). Peritubular capillary endothelial cells were sorted using a FACSaria (BD Pharmingen). Purified endothelial cells were cultured in medium as described above with addition of IFN-γ in permissive conditions. Cells were cultured for 8 d without IFN-γ; in this period, expression of SV40 is lost (data not shown) and cells differentiate into their original phenotype.

Differentiated endothelial cells were detached from the culture system using 1 mM EDTA. Endothelial cells were characterized using antibodies directed against CD31 (Abcam, Cambridge, UK), von Willebrand factor (vWF, F8; DAKO), and CD34-PE and CD44-PE (both from BD Pharmingen). Secondary antibodies goat anti-rat–FITC (CD31-staining) and goat anti-rabbit–FITC (vWF staining) were obtained from DAKO.

Peritubular Capillary Endothelial Cell Apoptosis

Fully differentiated $CD44^{+/+}$ and $CD44^{-/-}$ peritubular capillary endothelial cells were incubated at low serum (0.5% FCS) for 16 h followed by a 24-h incubation with TGF-β1 (R&D Systems) or TSP-1 (Sigma) with or without addition of 50 μg/ml HA (derived from rooster comb; Sigma). Apoptosis of endothelial cells was determined by flow cytometry, using Annexin-V–FITC (BD Pharmingen) and 7-amino-actinomycin D (7-AAD) (Via-Probe; BD Pharmingen). Annexin-V–positive cells but negative for 7-AAD were considered apoptotic.

Statistical Analyses

Multiple comparisons were performed using a repeated measures ANOVA corrected by a Bonferroni *post hoc* test.

Results

De Novo *CD44 Expression on Peritubular Endothelial Cells during UUO*

Under normal conditions, CD44 is not expressed in the kidney except on passenger leukocytes. Already 3 d after UUO, we observed *de novo* expression of CD44 on the capillary network

of $CD44^{+/+}$ mice, as determined by double immunostaining for CD34 and CD44 (Figure 1A). Flow cytometric analysis of a single cell suspension prepared from renal tissue 3 and 7 d after UUO confirmed CD44 expression on CD34-positive capillary endothelial cells (Figure 1B). In sham-operated kidneys, the percentage of CD44-positive endothelial cells (CD34-labeled cells) was approximately 5%. The number of CD44-positive cells increased after UUO to reach approximately 30% after 3 d (data not shown) and 40% after 7 d.

CD44 Deficiency Decreases Capillary Network Degeneration

In progressive renal diseases, development of fibrosis is associated with capillary network regression. To unravel the role of CD44 on peritubular capillary endothelial cells during UUO, we compared the capillary network in $CD44^{+/+}$ and $CD44^{-/-}$ mice.

Seven days after UUO, regression of the capillary network in the $CD44^{+/+}$ mice was apparent, whereas the capillary network of the $CD44^{-/-}$ mice was still preserved (Figure 2). Degeneration of the capillary network correlated to the development of fibrosis ($r = -0.52$, $P < 0.001$) as determined by CD34 staining and hydroxyproline contents (12).

Degeneration of the capillary network could be due to decreased proliferation and/or increased apoptosis of the capillary endothelial cells in $CD44^{+/+}$ as compared with $CD44^{-/-}$. We observed a sharp rise in the number of proliferating endothelial cells at day 3 after UUO, which, however, was similar in both phenotypes (Figure 3A). Apoptosis of endothelial cells increased after UUO in both phenotypes. However, 7 and 14 d after UUO, we observed a two-fold increase in the number of apoptotic peritubular capillary endothelial cells in $CD44^{+/+}$ compared with $CD44^{-/-}$ kidneys (Figure 3B).

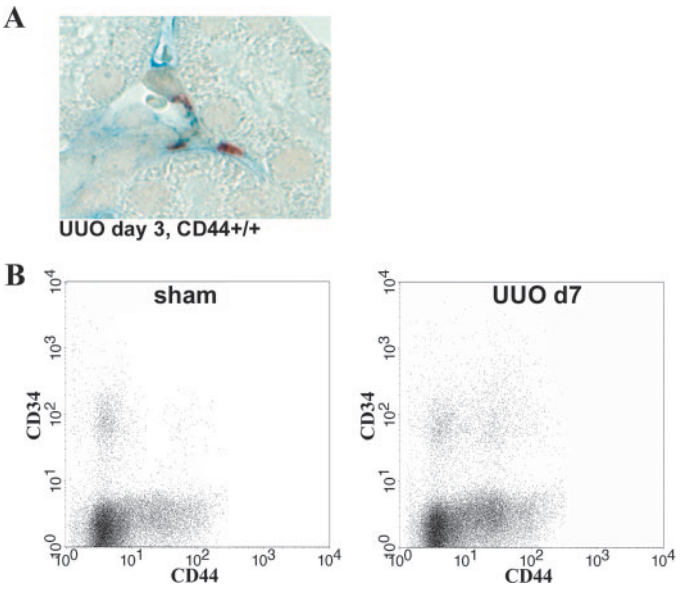

Figure 1. Capillary endothelial cells express CD44 after injury. Double immunostaining for CD44 (red) and CD34 (blue) reveals coexpression as soon as 3 d after unilateral ureteral obstruction (UUO; A). Flow cytometric analysis of endothelial cells (CD34 positive) in total kidney cell suspensions revealed CD44 expression after UUO (B). Only 5% double labeled (CD34 and CD44), of the total CD34-positive cells, expressed CD44 after sham operation. Seven days after UUO, 40% of the CD34-positive cells expressed CD44. Magnification, ×600 in A.

Similar Expression Levels of VEGF and TSP-1 in $CD44^{+/+}$ and $CD44^{-/-}$ Obstructed Kidneys

Because VEGF has mitogenic and protective functions on endothelial cells, we determined VEGF expression during UUO. VEGF was constitutively expressed on tubular epithelial cells. During UUO, VEGF expression was still observed on intact tubuli (Figure 4), but dilated tubuli failed to express VEGF. No differences were detected between $CD44^{+/+}$ and $CD44^{-/-}$ obstructed kidneys.

TSP-1 is a very potent proapoptotic molecule for capillary endothelial cells. TSP-1 expression was confined to the tubulointerstitial area in UUO. Despite significant differences in apoptotic rate of peritubular capillary endothelial cells, digital image analysis revealed no differences in expression of TSP-1 between $CD44^{+/+}$ and $CD44^{-/-}$ mice (Figure 5). To unravel the pathogenic mechanism of endothelial CD44 expression, we established $CD44^{+/+}$ and $CD44^{-/-}$ peritubular capillary endothelial cell lines.

Characterization of Peritubular Capillary Endothelial Cells

After 8 d of differentiation at 37°C without IFN-γ, cells no longer expressed SV40 or PCNA (data not shown). Exposure to 10 ng/ml TNF-α for 4 h induced high levels of VCAM-1 (Figure 6A) and E-selectin (Figure 6B). The endothelial marker CD34 (Figure 6C) was not expressed by *in vitro* cultured capillary endothelial cells. vWF (Figure 6D) and CD31 (Figure 6E) both were expressed constitutively as determined by flow cytometry. Expression patterns of VCAM-1, E-selectin, CD34, vWF, and CD31 were comparable in $CD44^{+/+}$ and $CD44^{-/-}$ cell lines. As expected, $CD44^{-/-}$ endothelial cells expressed no CD44, whereas $CD44^{+/+}$ cells express high levels of CD44 (Figure 6F).

CD44-HA Interaction Enhances TGF-β1– but not TSP-1–Induced Apoptosis in Capillary Endothelial Cells

The viability and apoptotic rate of fully differentiated, nondividing $CD44^{+/+}$ and $CD44^{-/-}$ endothelial cells were similar under differentiation conditions. Addition of HA had no effect on the percentage of apoptosis of $CD44^{+/+}$ and $CD44^{-/-}$ endothelial cells. Cells were stimulated for 24 h using different concentrations of TGF-β1 with or without addition of 50 μg/ml HA (Figure 7A). Various concentrations of TGF-β1 failed to induce apoptosis. However, when HA was added simultaneously to the cell cultures, the rate of apoptosis of endothelial cells that expressed CD44 increased but not in CD44-deficient endothelial cells. This observation suggests that CD44-HA ligation is required for TGF-β1 induction of apoptosis in endothelial cells.

As expected, TSP-1 induced apoptosis in a dose-dependent manner (Figure 7B). However, in contrast to TGF-β1, CD44 or interaction of CD44 with HA had an effect only on TSP-1–

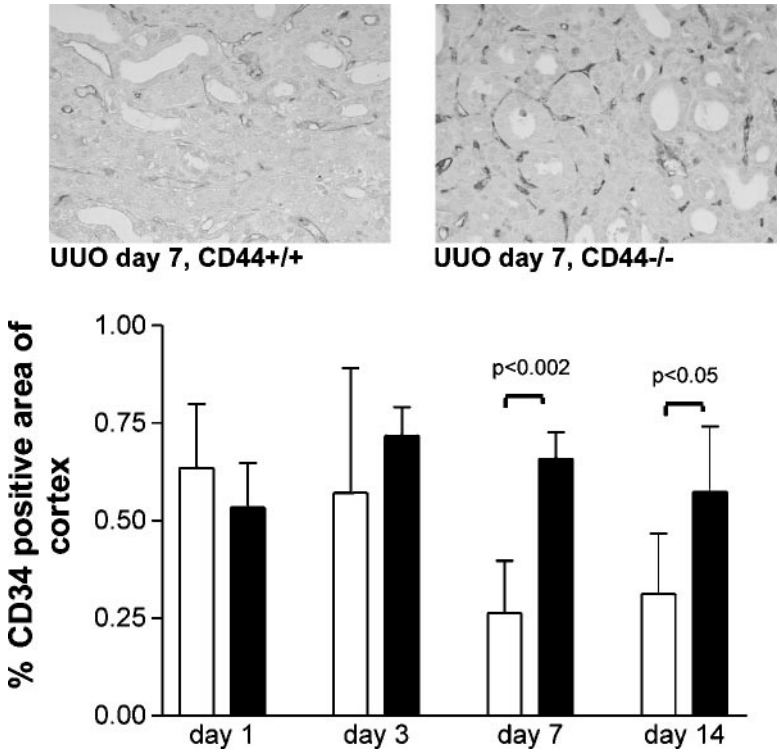

Figure 2. CD44 deficiency prevents capillary regression. Immunostaining for CD34 revealed degeneration of the capillary network in $CD44^{+/+}$ (left) mice (□) but not in $CD44^{-/-}$ (right) mice (■); staining is quantified by digital analysis. Data are presented as mean ± SEM; $n = 6$. Magnification, ×100.

induced apoptosis at 20 ng/ml. At higher concentrations, this effect was no longer observed.

Discussion

A cascade of events is initiated during the progression of tubulointerstitial lesions, including release of cytokines/chemokines and growth factors, expression of adhesion molecules, inflammatory infiltrate, renal epithelial cell damage, accumulation of myofibroblasts, capillary network degeneration, and finally fibrosis. CD44 is upregulated in various diseases, including experimental nephropathies and human renal diseases (13–17). CD44 therefore may be considered as a regulatory molecule in the cascade that leads to tubulointerstitial damage. Using a mouse model of chronic obstructive nephropathy, we showed that CD44 protects tubuli from injury but also induces the development of fibrosis (12).

In this study, we determined the role of CD44 in the degeneration of the capillary network. Loss of peritubular and glomerular capillaries is correlated with the severity of renal disease (19,20). Ohashi *et al.* (22) documented that capillary network regression resulted from increased endothelial cell loss and impaired regeneration. As documented in other diseases (10,13), we show that the capillary endothelial cells express CD44 after renal injury. As expected, the capillary network degenerated in $CD44^{+/+}$ after UUO. Remarkably, the capillary network was well preserved in $CD44^{-/-}$ obstructed kidneys. The degree of proliferation of peritubular capillary endothelial cells was comparable in mice of both phenotypes. However, the number of apoptotic endothelial cells was less in $CD44^{-/-}$ compared with $CD44^{+/+}$, which may contribute to the preservation of the capillary network in $CD44^{-/-}$ obstructed kidneys. Many reports emphasize an important role for VEGF in proliferation of endothelial cells and preservation of the capillary network (20–22). Activation of CD44 by HA or activating antibodies stimulates VEGF production *in vitro* in human vascular endothelial cells (31). However, no differences in VEGF expression could be detected in $CD44^{+/+}$ and $CD44^{-/-}$ obstructed kidneys. This is in accordance with the comparable number of proliferating endothelial cells in $CD44^{+/+}$ and $CD44^{-/-}$ mice. A potent inducer of apoptosis for endothelial cells is TSP-1 (24,25). Therefore, we analyzed expression of TSP-1. No difference in expression of TSP-1 in mice of both genotypes that may explain the difference observed in endothelial cell apoptosis was found, yet CD44 may enhance signaling of TSP-1 because TSP-1, like hepatocyte growth factor, is a heparan sulfate–binding growth factor (24), the signaling of which can be enhanced in the presence of CD44v3 (8,12). However, using conditionally immortalized capillary endothelial cell lines derived from $CD44^{+/+}$ or $CD44^{-/-}$ kidneys, we were unable to show a clear facilitating role for CD44 in TSP-1–induced apoptosis of capillary endothelial cells. Interaction of HA with CD44 was only at 20 ng/ml TSP-1 able to increase apoptosis in $CD44^{+/+}$ endothelial cells, suggesting a possible role for CD44 in TSP-1 signaling. Lowering TSP-1 concentrations below 20 ng/ml TSP-1 resulted in background apoptosis levels.

Besides TSP-1, TGF-β1 can induce apoptosis in endothelial

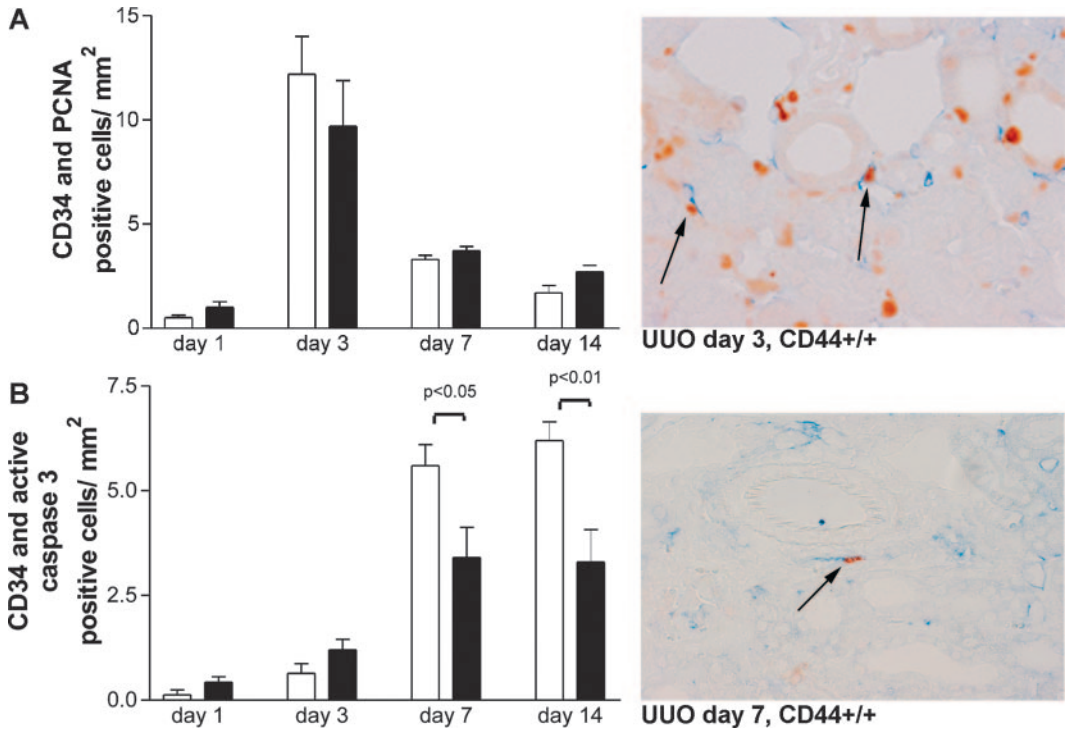

Figure 3. Increased endothelial apoptosis in $CD44^{+/+}$ compared with $CD44^{-/-}$ obstructed kidneys. (A) Double immunostaining for proliferating cell nuclear antigen (PCNA; red) and CD34 (blue; as indicated by arrows) revealed no differences in endothelium proliferation in $CD44^{+/+}$ kidneys (□) and $CD44^{-/-}$ kidneys (■) after UUO; data are presented as double-positive cells/mm^2. (B) Double staining for active caspase-3 (red) and CD34 (blue; as indicated by arrow) revealed increased apoptosis in $CD44^{+/+}$ (□) compared with $CD44^{-/-}$ (■) of peritubular endothelial cells; data are presented as double-positive cells/mm^2. All data are presented as mean ± SEM; $n = 6$. Magnification, ×400.

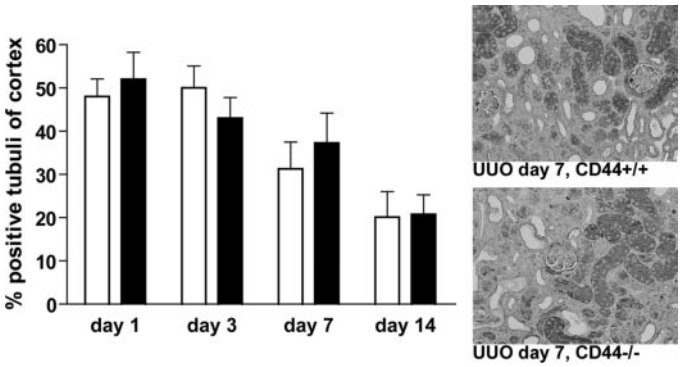

Figure 4. Vascular endothelial growth factor (VEGF) expression after UUO. Immunostaining for VEGF revealed comparable expression in $CD44^{+/+}$ (top) kidneys (□) and $CD44^{-/-}$ (bottom) kidneys (■). Data are presented as mean ± SEM; $n = 6$. Magnification, ×200.

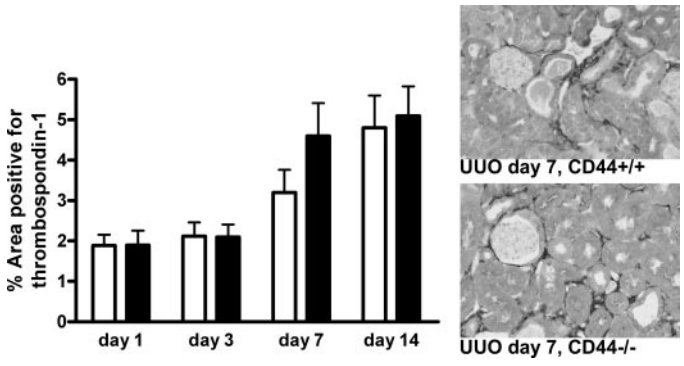

Figure 5. Thrombospondin-1 (TSP-1) expression after UUO. Immunostaining for TSP-1 revealed interstitial expression of TSP-1. Comparable expression was observed in $CD44^{+/+}$ (top) kidneys (□) and $CD44^{-/-}$ (bottom) kidneys (■). Data are presented as mean ± SEM; $n = 6$. Magnification, ×300.

cells (32,33). Previously, we showed that in our model, TGF-β1 is highly expressed (12). Addition of high concentrations of TGF-β1 was only moderately capable of inducing apoptosis in capillary endothelial cells, but a synergetic effect of HA was observed on TGF-β1–induced endothelial cell apoptosis. The potentiation of TGF-β1 proapoptotic activity was observed only in capillary endothelial cells that expressed CD44. This suggests that ligation of CD44-HA facilitates TGF-β1–induced apoptosis in peritubular capillary endothelial cells. Effects of CD44-HA interactions in TGF-β1 signaling were described previously in a tumor cell line (11) and also in tubular epithelial cells (12). Bourguignon *et al.* (11) showed that CD44 forms a complex with TGF-β1 receptor I and to a lesser extent TGF-β1 receptor II. Stimulation of CD44 with HA results in an increased threonine/serine kinase activity, resulting in increased smad2/3 phosphorylation. In agreement, we showed *in vivo* in $CD44^{+/+}$

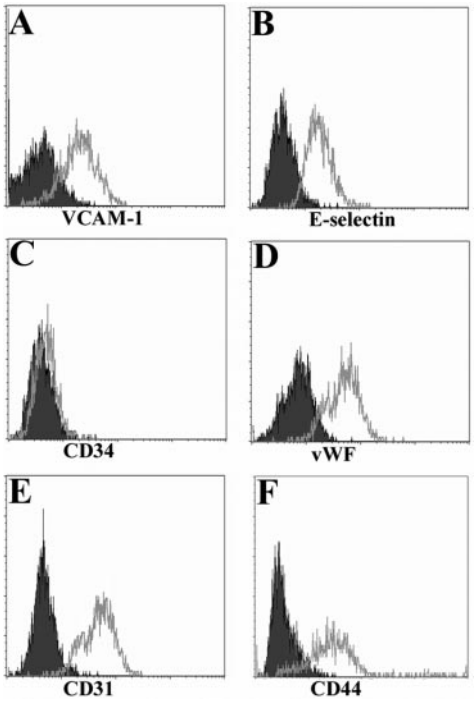

Figure 6. Peritubular capillary endothelial cell characterization. Flow cytometric analysis on fully differentiated peritubular capillary endothelial cells, 4 h after TNF-α stimulation (gray line), revealed increased expression of vascular cellular adhesion molecule-1 (VCAM-1; A) and E-selectin (B), compared with nonstimulated cells (filled area). Endothelial cells *in vitro* do not express CD34 (C); gray line represents CD34 labeled cells, and filled area is the control isotype antibody. Endothelial cells express von Willebrand factor (vWF; D) and CD31 (E); gray line represents specific antibody labeled cells, and filled are is the control isotype antibody. $CD44^{+/+}$ endothelial cells (gray line) express CD44; $CD44^{-/-}$ cells (filled area) do not express CD44 (F).

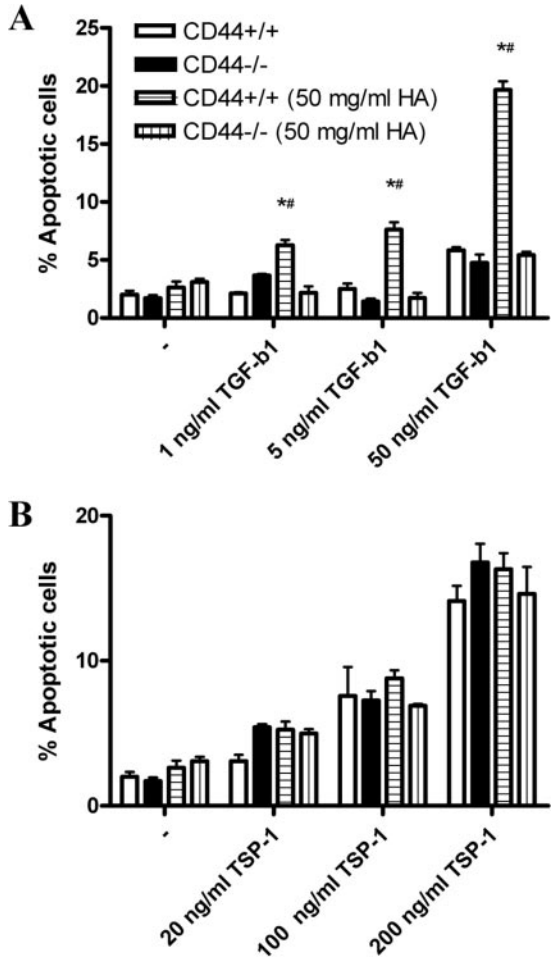

Figure 7. TGF-β1– but not TSP-1–induced apoptosis is enhanced by CD44–hyaluronic acid (HA) interaction. Fully differentiated capillary endothelial cells were stimulated for 24 h with TGF-β1 or TSP-1 with or without addition of 50 μg/ml HA. Apoptosis was determined by flow cytometry. TGF-β1 was capable of inducing apoptosis only in $CD44^{+/+}$ cells after CD44-HA ligation (A). TSP-1 induced apoptosis in endothelial cells independent of the receptor-ligand complex, CD44-HA (B). □, $CD44^{+/+}$; ■, $CD44^{-/-}$; ▤, $CD44^{+/+}$ with HA; and ▥, $CD44^{-/-}$ with HA; data are presented as mean ± SEM; $n = 3$. $^{*}P < 0.05$ ($CD44^{+/+}$ with HA *versus* $CD44^{+/+}$); $^{\#}P < 0.05$ ($CD44^{+/+}$ with HA *versus* $CD44^{-/-}$ with HA).

and $CD44^{-/-}$ mice (12) that the absence of CD44 resulted in decreased activation of the smad2/3 proteins. This decreased TGF-β1 signaling in turn prevented the development of fibrosis in $CD44^{-/-}$ mice. It is generally considered that TGF-β1 is the major growth factor involved in transdifferentiation of tubular epithelial cell (TEC) into myofibroblasts (34). Whereas myofibroblasts generally are thought to derive from transdifferentiation of TEC and from resident fibroblasts, increasing evidence has been gathered that myofibroblasts can also derive from hematopoietic cells (35). We observed no transdifferentiation of endothelial cells into myofibroblasts (data not shown). Although it is well established that myofibroblasts play a major role in the development of renal fibrosis, the relative contribution of the different "types" of myofibroblasts is still a matter of debate.

Previous reports implicated CD44 in Fas-induced apoptosis; however, these reports are contradictory. One study showed that CD44 stimulation downregulates Fas expression and Fas-mediated apoptosis of pulmonary cancer cells (36), but another study described that CD44 markedly enhanced Fas expression and subsequently Fas-induced apoptosis of these cells (37). These contradictory observations prompted us to investigate Fas expression in our model by immunostaining. No difference in Fas expression was observed between $CD44^{+/+}$ and $CD44^{-/-}$ UUO kidneys (data not shown).

Although CD44 increases the number of apoptotic endothelial cells, we showed previously that in the same model, expression of CD44 on TEC decreases apoptosis of TEC (12). This observed paradoxic effect of CD44 on TEC and endothelial cells may be related to the multiple modifications of CD44, *e.g.*, alternative splicing; glycosylation; addition of keratin, heparan, or chondroitin sulfate side chains. This modifies the properties of CD44 and therefore may contribute to enhancement of cer-

tain growth factor signaling, whereas it inhibits others. Although the cellular processes that lead to alternative splicing still are poorly understood, expression of CD44 isoforms seems to be tissue specific. Therefore, the enhancement or inhibition of growth factor signaling by CD44 may also be tissue specific.

Although we show a clear role for CD44 together with TGF-β1 in the degeneration of the capillary network, this does not exclude involvement of other growth factors. Moreover, it remains unclear whether development of fibrosis is dependent on capillary degeneration or *vice versa*. This study together with that of Kang *et al.* (20,21) shows that capillary degeneration and development of fibrosis are associated with each other. Blocking interaction between HA and CD44 therefore may be a potential therapeutic opportunity to prevent progression of chronic renal diseases. Not only may the degeneration of the capillary network be prevented but also the development of fibrosis (12).

Conclusion

This report shows that expression of CD44 in the injured kidney contributes to the degeneration of the capillary network, at least in part through enhanced TGF-β1–induced apoptosis of endothelial cells.

Acknowledgments

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