Bone Morphogenic Protein-7 Inhibits Monocyte-Stimulated TGF-β1 Generation in Renal Proximal Tubular Epithelial Cells

Xiao Liang Zhang,* Wisam Selbi,* Carol de la Motte,† Vincent Hascall,‡ and Aled O. Phillips*

*Institute of Nephrology, University of Wales College of Medicine, Heath Park, Cardiff Wales, United Kingdom; and Departments of †Colorectal Surgery and ‡Biomedical Engineering, The Cleveland Clinic Foundation, Cleveland, Ohio

It has been demonstrated that bone morphogenic protein-7 (BMP-7) stimulates formation of hyaluronan (HA)-based cables on the cell surface of renal proximal tubular cells and that these cables mediate monocyte binding. Furthermore, interaction of monocytes with proximal tubule cell (PTC) surface intracellular adhesion molecule (ICAM) stimulates the synthesis of TGF-β1. This study examined the effect of BMP-7 on monocyte-stimulated TGF-β1 synthesis under conditions of basal and stimulated ICAM expression. Monocyte (U937 cells)-dependent stimulation of TGF-β1 promoter activity and protein synthesis was reduced by addition of BMP-7 for 24 h before addition of U937 cells. Removal of cell surface HA or inhibition of monocyte interaction with HA using antibody to CD44 prevented this effect of BMP-7. These data suggest that BMP-7 enhances HA-dependent binding and reduces ICAM-dependent binding, which is known to stimulate TGF-β1 synthesis. This hypothesis was examined further by stimulation of PTC ICAM expression by TNF-α. After TNF-α stimulation, monocyte-dependent TGF-β1 synthesis increased. This was abrogated by inhibition of ICAM-CD18 interactions. TNF-α stimulation alone did not increase TGF-β1 synthesis. TNF-α stimulation of PTC in the presence of BMP-7 failed to increase monocyte-dependent TGF-β1 stimulation. Although stimulation of PTC by BMP-7 alone decreased cell surface ICAM expression, it did not affect TNF-α–induced ICAM expression. The effect of BMP-7 on TGF-β1 synthesis in TNF-α–stimulated cells was abrogated by disruption of CD44–HA interactions, suggesting that it was due to increased monocyte binding to HA on the cell surface.


Received May 18, 2004. Accepted September 17, 2004.

Published online ahead of print. Publication date available at www.jasn.org.

Address correspondence to: Dr. Aled O. Phillips, Institute of Nephrology, University of Wales College of Medicine, Heath Park, Cardiff CF14 4XN. Phone: 44-2920-748411; Fax: 44-2920-748470; E-mail: PhillipsAO@cf.ac.uk

Copyright © 2005 by the American Society of Nephrology

ISSN: 1046-6673/1601-0079
HA-mediated binding of monocytes represents an antifibrotic mechanism that limits the generation of TGF-β1 and that this is regulated by BMP-7. Specifically, we examined the effect of BMP-7 on monocyte-stimulated TGF-β1 synthesis under conditions of basal and stimulated cell surface ICAM expression.

Materials and Methods

Reagents and Antibodies
All tissue culture plastics were obtained from Becton Dickinson Ltd. (Oxford, UK). Reporter Lysis Buffer and Bright-Glo Luciferase Assay System were purchased from Promega (Madison, WI). Fugene6 transfection reagent was bought from Roche (Indianapolis, IN). 51Cr as sodium chromate was obtained from Amersham Biosciences (Chalford St. Giles, UK). Other reagents and sources were as follows: testicular hyaluronidase (H3884; Sigma-Aldrich, Poole, UK), recombinant human soluble ICAM-1 (R&D Systems Europe Ltd., Abingdon, UK), anti-human CD44 blocking mAb BU75 and anti-human CD18 blocking mAb (Ancell Corp, supplied by Qiogene-Alexis Ltd., Oxford, UK), antihuman IgG (whole molecule; Sigma), recombinant TGF-β1 and TNF-α (R&D Systems), and BMP-7 (Creative Biomolecules, Boston, MA).

Cell Culture
All experiments were done using HK-2 cells (American Type Culture Collection no. CRL-2190), which are human proximal tubular epithelial cells immortalized by transduction with human papilloma virus 16 E6/E7 genes (19). Previously, it has been demonstrated that HK-2 cells do not express BMP-7 mRNA, although analysis of receptor expression revealed transcripts for BMP receptors type IA and IB (20). Cells were cultured in DMEM/Ham’s F12 (Life Technologies, Paisley, UK) supplemented with 10% FBS (Biologic Industries Ltd., Cumbernauld, UK), t-glutamine, insulin, transferrin, sodium selenite, hydrocortisone, and HEPES (Sigma-Aldrich). Fresh growth medium was added to cells every 3 to 4 d until confluent. All experiments were done using cells at passage 30 or below, and cells were growth-arrested in serum-free medium for 48 h before use in experiments. All experiments were subsequently done in serum-free conditions.

U937 cells, originally derived from a human histiocytic lymphoma, were procured from the American Type Culture Collection (Rockville, MD). Their interactions with PTC in terms of ICAM and HA cables are routinely subcultured at a 1:5 ratio three times per week.

Analysis of TGF-β1 Transcriptional Activity
The TGF-β1 promoter-luciferase construct pGL3-TGFβ1 +11/−1362 was generated as described previously (21). The pSV-β-galactosidase control vector was purchased from Promega UK Ltd (Southampton, UK). For transfection of the reporter construct, 1.3 × 10⁶ HK-2 cells were seeded per 24-well plate and incubated overnight. This density of cells produced an approximately 70% confluent monolayer the following day. The cells then were transfected with 0.2 μg of plasmid pGL3-TGF-β1 +11/−1362 and 0.2 μg of pSV-β-galactosidase plasmid (to act as an internal control for transfection efficiency), using the mixed lipofection reagent FuGene 6 (Roche) at a ratio of 3 μl of FuGene to 1 μg of DNA in serum-free and insulin-free medium. Twenty-four hours after transfection, unstimulated U937 cells were added to the monolayer of HK-2 cells for up to 24 h. After lysis of the cells in Reporter Lysis Buffer (Promega UK Ltd), β-galactosidase activity was determined by colorimetric assay (β-Galactosidase Enzyme Assay System; Promega UK Ltd), and luciferase content was quantified by a glow-type luminescence assay (Bright-Glo; Promega UK Ltd).

TGF-β1 Protein Quantification
In all experiments, total TGF-β1 in the cell culture supernatants was measured by specific ELISA (R&D Systems). Active TGF-β1 is measured directly, and latent TGF-β1 can be measured indirectly after acid activation of samples. This assay has <1% cross-reactivity for TGF-β2 and TGF-β3.

Assay for Leukocyte Adhesion
U937 cell adhesion was measured as described previously (22). Briefly, HK-2 cells were grown on 24-well culture plates until confluent. On the day of assay, U937 cells (up to 70 × 10⁶ cells/ml) were labeled for 90 min at 37°C with 100 μCi of 51Cr as sodium chromate (Amersham Biosciences). The labeled cells were washed three times with serum-free culture medium, counted on a hemacytometer, and resuspended to 10⁶ viable cells/ml (as determined by Trypan blue dye exclusion). Incubation medium was removed from HK-2 cultures, and 10⁶ or 0.3 × 10⁶ monocytes were added to each well, depending on the experimental set up. The binding phase of the assay was done at 4°C for 1 h. All cultures were washed with cold medium before lysis by 1% Triton X-100. An aliquot was subsequently removed for quantification of radioactivity. The number of U937 cells bound per well was calculated from the initial specific activity (cp.m.cell). Spontaneous release of chromium from U937 cells in control incubations without HK-2 cells was typically <10%.

FACS Analysis
Cell surface expression of ICAM was assessed by FACS analysis. After detachment of HK-2 cell monolayers, the cells were incubated with R-phycoerythrin (R-PE)-conjugated mouse anti-human CD54 (ICAM-1) mAb (Becton Dickinson Ltd., Oxford, UK; 1:20 dilution) or mouse anti-human IgG Ab (BD PharMingen; 1:20 dilution) as a control, at saturating concentrations in FACS buffer (PBS, 10 mM EDTA [Sigma-Aldrich], 15 mM sodium azide [Fisher Scientific UK, Leicester, UK], and 5% BSA [Sigma-Aldrich; pH 7.35]) for 30 min at 4°C. After three washes in FACS buffer, the data were collected using a Becton Dickinson FACSCalibur 4Ca and analyzed using CellQuest Pro software.

Statistical Analyses
Statistical analyses were performed using SPSS version 11.0.2 software (SPSS Inc, Chicago, IL). Differences between groups were tested using ANOVA with the Bonferroni post hoc test used for multiple comparisons. P < 0.05 was considered to represent a significant difference. The data are presented as means ± SD of n experiments. For each individual experiment, the mean of duplicate determinations was calculated.

Results

BMP-7 Reduces Monocyte-Dependent TGF-β1 Generation
Previously, we demonstrated that BMP-7 stimulation of HK-2 cells increased HA-dependent monocyte binding. To determine the functional consequence of this, we examined the effect of BMP-7 on monocyte-driven TGF-β1 generation. HK-2 monolayers that were transiently transfected with the TGF-β1 promoter reporter construct were stimulated with BMP-7 (400 ng/ml) for 24 h before addition of 0.3 × 10⁶ unstimulated U937 cells at 37°C for an additional 24 h.

As described previously (4), addition of U937 cells to HK-2
cell monolayers led to an increase in TGF-β1 promoter activity (Figure 1A, lane 3). In contrast, when HK-2 cells were stimulated with BMP-7 before the addition of U937 cells, there was no increase in TGF-β1 promoter activity (Figure 1A, lane 4). TGF-β1 promoter activity after addition of BMP-7 alone (Figure 1A, lane 2) was no different from the effect of addition of serum-free medium alone (Figure 1A, lane 1). Similarly, quantification of TGF-β1 protein in the cell culture supernatant confirmed reduction in monocyte-driven TGF-β1 protein synthesis after BMP-7 treatment of HK-2 cell monolayers (Figure 1B). Addition of U937 cells to unstimulated HK-2 cells resulted in a 1.4-fold increase in TGF-β1 (383.6 ± 72.3 versus 544.5 ± 61.7 mean ± SD, pg/ml; n = 5; P < 0.01; Figure 1B, lanes 1 and 3). In contrast, there was no significant difference in TGF-β1 concentration in the supernatant between the control cells to which no U937 cells were added and that seen when U937 cells were added to BMP-7–treated monolayers (383.6 ± 72.3 versus 414.2 ± 38.2 mean ± SD, pg/ml; N = 5; Figure 1B, lanes 1 and 4).

Previously, we identified HA in cable-like structures that spanned several cell lengths that seem to be composed of coalescing bundles of thinner HA strands originating from neighboring cells. Furthermore, BMP-7 increased the formation of HA-based cables and was associated with an increase in HA in the pericellular and cell-associated fractions of HA, as assessed by analysis on Sephacryl S-500 of 3H-labeled HA (9). We therefore sought to examine the role of HK-2 cell surface HA in the modulation of TGF-β1 generation after stimulation by BMP-7. HK-2 cell monolayers that were transfected with the TGF-β1 reporter construct were stimulated with BMP-7 for 24 h. Subsequently, cell surface HA was removed by addition of testicular hyaluronidase before addition of U937 cells. Addition of U937 cells to hyaluronidase-treated HK-2 monolayers led to a significant increase in TGF-β1 promoter reporter construct activity both in unstimulated HK-2 cell monolayers and in monolayers that were treated with BMP-7 (Figure 2A, lanes 3 and 4). Importantly, the ability of BMP-7 to decrease monocyte-dependent TGF-β1 promoter activity was completely abolished by hyaluronidase treatment (Figure 2A, lane 4). Hyaluronidase treatment of the HK-2 cell monolayers, in the absence of added monocytes, did not influence TGF-β1 promoter activity (data not shown).

In addition to the removal of cell surface HA from the HK-2 cell monolayers, we used blocking antibodies to CD44 to delineate further the role of interactions between PTC HA and its receptor CD44 on the monocytes. In these experiments, U937 cells were incubated with anti-CD44 antibody at 37°C for 60 min before their addition to HK-2 cell monolayers. As with removal of cell surface HA, inhibition of HA–CD44 interaction using this protocol increased TGF-β1 promoter reporter activity both in unstimulated HK-2 cell monolayers and in monolayers that were treated with BMP-7 (Figure 2B, lanes 3 and 4). Addition of the U937 cells to the transfected HK-2 cells in the absence of the antibody prevented BMP-7–mediated reduction in TGF-β1 promoter activity after addition of U937 cells (Figure 2B, lanes 2 and 4). Addition of the anti-CD44 antibody to HK-2 cell monolayers did not influence TGF-β1 promoter activity (data not shown). In all experiments, changes in TGF-β1 promoter activity were mirrored by corresponding changes in TGF-β1 protein concentration in the cell culture supernatants (Figure 3). Reduction in TGF-β1 generation after addition of U937 cells to BMP-7–treated monolayers was reversed by dis-
ruption of CD44–HA interactions either by using hyaluronidase treatment to remove cell surface HA (Figure 3, lanes 2 and 3) or by directly inhibiting the interaction using CD44-blocking antibody (Figure 3, lanes 2 and 4).

**TNF-α Increases Monocyte-Dependent TGF-β1 Generation**

We previously demonstrated that hyaluronidase treatment of unstimulated HK-2 cell monolayers resulted in a significant increase in ICAM-dependent monocyte binding and monocyte-dependent TGF-β1 generation. The results presented above, therefore, would suggest that BMP-7–stimulated HA-dependent monocyte binding competes with cell surface adhesion molecule binding of monocytes.

It was reported previously that BMP-7 may antagonize the effect of the proinflammatory cytokine TNF-α, which is a well-established regulator of adhesion molecules. We therefore sought to examine the hypothesis that BMP-7 may also antagonize the effect of TNF-α in our experimental system.

To examine the effects of BMP-7 on proinflammatory cytokine-driven responses, we first examined the effect of TNF-α on ICAM-dependent monocyte binding and ICAM expression. Monocyte binding after TNF-α stimulation was quantified by addition of radiolabeled U937 cells to HK-2 cell monolayers that were pretreated with TNF-α (10 ng/ml) for 24 h (Figure 4A). Pretreatment of HK-2 cells with TNF-α resulted in a 2.2-fold increase in bound radioactivity (Figure 4A, lane 2). This increase in bound radioactivity was abrogated by incubation of U937 cells with anti-CD18 antibody, suggesting that increased monocyte binding was dependent on CD18–ICAM interactions (Figure 5A, lane 3). In parallel experiments, increased cell surface expression of ICAM after stimulation by TNF-α was confirmed by FACS analysis (Figure 4B).

The relationship between TNF-α-stimulated monocyte binding and TGF-β1 generation was examined by stimulation of HK-2 cells, transfected with the TGF-β1 promoter reporter construct, with TNF-α for 24 h before addition of unstimulated U937 cells. Under these conditions, pretreatment with TNF-α resulted in a significant increase in luciferase activity compared with that seen after addition of U937 cells to untreated HK-2 cell monolayers (Figure 6A, lanes 2 and 3). In parallel experiments, pretreatment with TNF-α also led to a significant increase in TGF-β1 concentration in the cell culture supernatant after addition of U937 cells, compared with that seen after addition of U937 cells to untreated HK-2 cell monolayers (Figure 6B, lanes 1 and 2). Addition of either blocking antibody to CD18 or soluble ICAM to monolayers of HK-2 cells did not alter TGF-β1 promoter reporter activity (data not shown).

For demonstrating the relationship between TGF-β1 generation and TNF-α-induced ICAM expression, in a parallel set of experiments, the interaction between monocyte CD18 and HK-2 cell surface ICAM was inhibited by using either blocking antibody to CD18 or soluble ICAM. U937 cells were incubated with either anti-CD18 antibody or soluble ICAM for 60 min at 37°C before their addition to HK-2 cells that were stimulated with TNF-α. The effect of TNF-α stimulation resulting in increased TGF-β1 promoter activity, and also TGF-β1 protein concentration in the cell culture supernatant was inhibited by...
blocking CD18–ICAM interaction either by addition of U937 cells to HK-2 monolayers in the presence of soluble ICAM (Figure 6A, lane 4, and B, lane 3) or by treatment of U937 cells with CD18 blocking antibody (Figure 6A, lane 5, and B, lane 4) but not by addition of 10 μg/ml IgG (data not shown).

Having demonstrated the role of CD18–ICAM–dependent binding in monocyte-stimulated TGF-β1 generation after TNF-α stimulation, we next examined the role of cell surface HA in these experiments. HK-2 monolayers were stimulated with TNF-α before addition of testicular hyaluronidase and subsequent addition of U937 cells. This resulted in a significant increase in TGF-β1 promoter activity (Figure 6A, lane 6) and in protein synthesis (Figure 6B, lane 5) compared with that observed when U937 cells were added to TNF-α–stimulated HK-2 monolayers that had not been treated with hyaluronidase. This is consistent with the hypothesis that cell surface HA in both the unstimulated HK-2 cells and the TNF-α–stimulated cells reduces monocyte–ICAM interactions and the resultant generation of TGF-β1. That enhanced TGF-β1 generation after removal of cell surface HA was indeed the consequence of monocyte CD18–HK-2 ICAM interactions was demonstrated by blocking these interactions using either anti-CD18 antibody or soluble ICAM. In hyaluronidase-treated HK-2 cells, increased TGF-β1 promoter activity and protein synthesis were abrogated by addition of U937 cells to HK-2 monolayers in the presence of soluble ICAM (Figure 6A, lane 7, and B, lane 6) or by treatment of U937 cells with CD18 blocking antibody (Figure 6A, lane 8, and B, lane 7).

**BMP-7 Antagonism of TNF-α–Primed, Monocyte-Dependent TGF-β1 Synthesis**

For examining the effect of BMP-7 on TNF-α–stimulated, monocyte-dependent TGF-β1 generation, HK-2 cells that were transfected with the TGF-β1 promoter-luciferase reporter construct were stimulated by TNF-α in the presence of BMP-7 for 24 h before addition of unstimulated U937 cells. Co-stimulation with both factors resulted in significant attenuation in relative luciferase activity after addition of U937 cells, compared with stimulation with TNF-α alone (Figure 5A, lanes 3 and 4). In parallel experiments, co-stimulation of confluent monolayers of growth-arrested HK-2 cells by both TNF-α and BMP-7 for 24 h before addition of U937 cells resulted in a significant reduction TGF-β1 protein concentration measured in the supernatant collected from cells that were stimulated with TNF-α alone (Figure 5B, lanes 3 and 4). Two possible mechanisms for attenuation of the effect of TNF-α were explored, namely antagonism of TNF-induced ICAM expression directly reducing interaction of U937 cells with HK-2 cell ICAM, and BMP-7–stimulated HA cable formation indirectly reducing U937 cell interaction with HK-2 cell ICAM.

Stimulation of confluent monolayers of HK-2 cells with BMP-7 under serum-free conditions led to a reduction in cell surface expression of ICAM at 24 h as assessed by FACS analysis (Figure 7A). In contrast to stimulation with BMP-7 alone, BMP-7 did not influence TNF-α–induced ICAM-1 expression. Cell surface expression of ICAM-1 was examined by FACS analysis after stimulation with either TNF-α alone for 24 h or TNF-α in the presence of BMP-7. Cell surface ICAM-1 expression was identical under these conditions (Figure 7B).

As attenuation of TNF-α–stimulated, monocyte-dependent TGF-β1 generation was unrelated to an effect of BMP-7 on ICAM-1 expression, we postulated that its effect was related to increased monocyte binding to cell surface HA cables, which in turn resulted in a reduction in ICAM-1–dependent monocyte binding. For testing this hypothesis, HK-2 cells that were transfected with the TGF-β1 promoter reporter construct were co-stimulated with TNF-α and BMP-7 for 24 h. Subsequently, cell surface HA was removed by addition of bovine testicular hyaluronidase (final concentration 200 μg/ml) to the HK-2 monolayer at 37°C for 5 min before addition of unstimulated U937 cells. Removal of cell surface HA by this method reversed the inhibitory effect of BMP-7 on TNF-α–stimulated monocyte-dependent TGF-β1 promoter activity (Figure 8A, lane 2 versus 3).

In parallel experiments, removal of cell surface HA after co-stimulation of confluent monolayers of HK-2 cells with both BMP-7 and TNF-α reversed the inhibitory effect of BMP-7 on TGF-β1 protein generation after addition of un-
stimulated U937 cells (Figure 8B, lane 2 versus 3). To demonstrate that the restoration of TNF-α-induced monocyte-dependent stimulation of TGF-β1 generation after hyaluronidase treatment was due to CD18–ICAM interaction, we performed experiments using either blocking antibody to CD18 or soluble ICAM. HK-2 cell monolayers were co-stimulated with BMP-7 and TNF-α, and cell surface HA was removed as described above. Subsequently, U937 cells were incubated with either 10 μg/ml anti-CD18 antibody or soluble ICAM for 1 h at 37°C before addition to the HK-2 cell monolayer. Under these experimental conditions, the increase in TGF-β1 promoter activity and protein synthesis,
which was observed after removal of cell surface HA alone, was attenuated (Figure 8, lanes 4 and 5).

**Discussion**

Renal proximal tubular epithelial cells play a key role in regulating the onset and progression of interstitial fibrosis. TGF-β1 is a relatively ubiquitous cytokine that functions in an autocrine or paracrine manner to elicit a multiplicity of effects, principally related to cell growth and extracellular matrix accumulation, that has been implicated widely in the pathogenesis of tissue fibrosis (23). In renal disease, it has been implicated in directing fibrosis in both glomerular and interstitial compartments (24–28) and is postulated to be a major biologic signal that regulates the switch in tubular cell activities toward a profibrogenic phenotype (29,30). Much of our work to date has focused on the mechanism of its generation and its effect on proximal tubular cell phenotype and function.

It is now clear that the macrophage influx into the renal corticointerstitium is associated with progressive renal scarring in a wide range of disease states (31,32). We recently described a novel mechanism by which mononuclear leukocytes via their cell surface CD44 bind to pericellular HA cable-like structures on proximal tubular epithelial cells. Furthermore, we have demonstrated that stimulation of PTC with BMP-7 positively regulates this interaction (9). These observations are consistent with observations using colonic mucosal smooth muscle cells,

**Figure 6.** BMP-7 prevents TNF-α–enhanced monocyte-stimulated TGF-β1 promoter activity (A) and protein synthesis (B). HK-2 cells that were transfected with the TGF-β1 promoter-reporter construct were stimulated with 400 ng/ml BMP-7 or 10 ng/ml TNF-α either alone or in combination for 24 h before removal of BMP-7/TNF-α, washing of the monolayer with PBS, and addition of 0.3 × 10⁶ unstimulated U937 cells for an additional 24 h and subsequent determination of luciferase activity (A) or addition of 0.3 × 10⁶ unstimulated U937 cells for 48 h before quantification of TGF-β1 concentration in the cell culture supernatant by ELISA (B). Results represent mean ± SD; n = 6; *P < 0.05 versus the combination of TNF-α, BMP-7, and hyaluronidase treatment.

**Figure 7.** BMP-7 decreases expression of ICAM but does not affect TNF-α–stimulated cell surface ICAM-1 expression. (A) The effect of BMP-7 on cell surface expression of ICAM was determined by stimulation of confluent growth-arrested HK-2 monolayers by the addition of 400 ng/ml BMP-7 and analysis of ICAM by FACS analysis. In control experiments, serum-free medium alone was added to the HK-2 cell monolayer (green line). One representative experiment of four individual experiments is shown. (B) The effect of BMP-7 on TNF-α–stimulated ICAM induction was determined by stimulation of confluent growth-arrested HK-2 cells with 10 ng/ml TNF-α alone (shaded area) or 10 ng/ml TNF-α in the presence of 400 ng/ml BMP-7 (green line) for 24 h. Cell surface ICAM-1 expression was subsequently examined by FACS analysis. In control experiments, HK-2 cells were exposed to serum-free medium alone (red line). One representative experiment of four individual experiments is shown.
which also demonstrated binding of nonactivated leukocytes to HA cable structures (33). We have also demonstrated that monocyte interaction with ICAM expressed on the basolateral aspect of PTC stimulates TGF-β1 synthesis (4). In the current study, we examined the functional consequences of increased HA-dependent monocyte binding in response to BMP-7 and, more specific, how this affects TGF-β1 generation resulting from the interaction between monocytes and renal proximal tubular epithelial cells.

Our data demonstrate that BMP-7 reduced monocyte-stimulated TGF-β1 generation, which we previously demonstrated to be dependent on interaction of monocytes with ICAM on the cell surface of the epithelial cell. Mechanistically, we propose that this depends on increased binding of monocytes to BMP-7-stimulated HA cables extending away from the PTC surface. These HA cables subsequently prevent access of ICAM to its receptor CD18 on the PTC surface (Figure 9). This assumption is supported by the observation that treatment of BMP-7-stimulated cell monolayers with hyaluronidase, which removes cell surface HA, normalized monocyte-dependent TGF-β1 synthesis. Although we cannot completely rule out the possibility that these results may be the summation of two opposing effects, our previous results demonstrating stimulation of HA cable-dependent binding and its abrogation by hyaluronidase treatment makes this unlikely. Furthermore, inhibition of HA receptor (CD44) function of the monocytic cells before their addition to BMP-7-stimulated monolayers also prevented a reduction in TGF-β1 generation.

In the adult, BMP-7 is predominantly expressed in distal renal tubular cells, where it is postulated to maintain the differentiated phenotype of tubular cells through autocrine or paracrine pathways (13). It was demonstrated previously that BMP-7 is not expressed by PTC (and particularly HK-2 cells), although they express BMP receptors, specifically Alk3 and Alk6 (20). From these data, it was suggested that BMP-7 is produced in the glomerulus and subsequently travels to the proximal tubule, where it exerts its biologic effects. In experimental models of renal disease, expression of BMP-7 in the kidney is decreased. Furthermore, the expression of its receptors on PTC also decreases, whereas the expression of a natural BMP-7 antagonist, gremlin, is increased, effects that at least in part are mediated by TGF-β1 (15). This suggests that chronic renal injury may represent a situation of relative BMP-7 deficiency. Recent in vivo and in vitro studies suggest that BMP-7 counteracts TGF-β1-mediated alteration in PTC phenotype, favoring retention of an epithelial cell phenotype and inhibiting epithelial-to-mesenchymal transition (34). This resulted from a direct Smad-dependent counteraction of the TGF-β1 signaling pathway by BMP-7, which reversed chronic renal injury. It is interesting that although these data suggest that BMP-7 may modify PTC response to TGF-β1, at least in vitro, BMP-7 does not directly affect expression of TGF-β1 (15). Our observations
are consistent with the hypothesis that loss of BMP-7 activity per se may be profibrogenic and offers a novel mechanism by which a relative deficiency of BMP-7 indirectly enhances TGF-β1 generation as a result of the interaction between infiltrating and resident cells.

The proinflammatory cytokine TNF-α has previously been identified as a modulator of HA-mediated interaction between monocytes and endothelial cells. More specifically, TNF-α stimulation of microvascular endothelial cells increased HA expression and CD44-dependent adhesion of T cells under both non-static shear and laminar flow conditions (35). This interaction is facilitated by TNF-α–mediated posttranslational modification of CD44 (36). In contrast, our results suggest that in proximal tubular epithelial cells, TNF-α did not enhance HA-dependent binding but rather increased the expression of cell surface ICAM-1 and subsequent monocyte-dependent TGF-β1 generation. These observations therefore suggest a cell-specific function of HA–monocyte interaction and are consistent with the hypothesis that the HA-dependent monocyte binding dampens the inflammatory/fibrotic response in renal PTC, whereas in endothelial cells, such an interaction may facilitate localization of emigrating cells at the vascular bed within sites of inflammation.

The demonstration of a relative deficiency of BMP-7 associated with renal disease has prompted many investigators to examine its potential therapeutic effect in ameliorating renal injury. In models of renal ischemia, infusion of BMP-7 reduces severity of renal injury and suppresses inflammation by down-regulation of intercellular adhesive molecules (16). Interstitial inflammation and fibrogenesis associated with unilateral ureteral obstruction are also prevented in vivo by administration of BMP-7 at the time of unilateral ureteral obstruction (17). In the same model, BMP-7 promoted maintenance of tubular epithelial integrity. In the streptozotocin model of diabetic nephropathy, BMP-7 therapy in vivo also markedly ameliorated glomerular pathology, decreased tubulointerstitial volume, and reduced proteinuria (18). Collectively, these studies suggest that BMP-7 may (1) limit the inflammatory cascade and subsequent fibrotic response by reducing the release of proinflammatory cytokines and chemokines (37), (2) maintain renal blood flow through actions on vasoactive peptides (17,37), and (3) antagonize the functional consequences of increased expression of TGF-β1 by disruption of postreceptor signaling events (34).

In vitro studies have demonstrated that BMP-7 suppresses both basal and TNF-α–stimulated expression of proinflammatory cytokines and chemokines in proximal tubular epithelial cells. This suggests that BMP-7 administration could mediate a reduction in the infiltration of macrophages into the renal inter-

Figure 9. The proposed mechanism by which BMP-7 decreases interaction of monocytes with adhesion molecules on proximal tubular cells. (A) Although ICAM is known to be expressed predominantly on the luminal aspect of PTC, under proinflammatory conditions (represented by TNF-α stimulation in this study), it may also be expressed and upregulated on the basal aspect of the cell. Under such conditions, infiltrating monocytes gain access to cell surface ICAM, which subsequently triggers the generation of a profibrotic response by stimulation of TGF-β1. In contrast, after stimulation by BMP-7 (B), PTC generate HA-based cable structures that preferentially bind infiltrating monocytes by engagement of monocyte CD44. This interaction subsequently prevents migration of monocytes onto the PTC surface, thus preventing their interaction with PTC ICAM.
stium in renal disease. Our data suggest an additional mechanism by which BMP-7 administration may ameliorate the fibrotic response. Although we were unable to demonstrate any effect of BMP-7 on TNF-α-induced ICAM-1 expression, BMP-7 did increase HA-dependent monocyte binding, and this attenuated monocyte-stimulated TGF-β1 synthesis. This therefore suggests that BMP-7, in addition to its previously reported role in reducing inflammatory cell infiltrate, is likely to reduce the profibrotic impact of any infiltrating inflammatory cells.

In summary, the data suggest that BMP-7 is an important regulator of the interaction between monocytes and proximal tubular epithelial cells. Furthermore, it provides a potential mechanism by which reduced expression of BMP-7 may contribute to the fibrotic response, and its administration may ameliorate renal injury.

Acknowledgments
This work was supported by a Grant from The National Kidney Research Fund. A.O.P. is supported by a GlaxoSmithKline Advanced Fellowship.

References
27. Jones CL, Buch S, Post M, McCulloch L, Liu E, Eddy AA: Renal extracellular matrix accumulation in acute puromy-