Parathyroid hormone-related protein promotes inflammation in the kidney with an obstructed ureter

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Parathyroid hormone-related protein (PTHrP) promotes fibrogenesis in the acutely damaged kidney. Considering the relation between fibrosis and inflammation, we studied transgenic mice that overexpress PTHrP in the proximal tubule. When unilateral ureteric obstruction was induced in these transgenic mice, we found that they had more renal tubulointerstitial damage, leukocyte influx, and expression of proinflammatory factors than their control littermates. Reversal of PTHrP constitutive overexpression in these transgenic mice or treatment of control mice with the PTHrP antagonist (7–34) decreased this inflammatory response. Losartan, which abolished obstruction-induced endogenous PTHrP upregulation, also decreased the latter response but less effectively in transgenic mice. The PTHrP fragment (1–36) induced nuclear factor-κB (NF-κB) activation and proinflammatory cytokine overexpression in mouse cortical tubule cells in culture as well as migration of the macrophage cell line Raw 264.7. All these effects were decreased by PTHrP (7–34) and NF-κB or extracellular signal-regulated kinase (ERK) activation inhibitors. Our findings suggest a critical role of PTHrP in the renal inflammatory process that results from ureteral obstruction and indicate that ERK-mediated NF-κB activation seems to be an important mechanism whereby PTHrP triggers renal inflammation.

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Parathyroid hormone (PTH)-related protein (PTHrP) is upregulated in various experimental nephropathies.[1] PTHrP overexpression correlates with the development of proteinuria in both diabetic mice and rats with tubulointerstitial damage after protein overload.[2,3] Moreover, recent studies indicate that PTHrP might contribute to tubular damage, renal function deterioration, and fibrosis after nephrotoxic injury in rodents.[4–6]

Inflammation plays a key role in progressive renal scarring and fibrogenesis in various kidney diseases.[7–10] Nuclear factor-κB (NF-κB)-related cytokines appear to have an important role in renal injury-associated inflammation.[11–14] This factor commonly consists of a heterodimer of one p50 subunit and one p65 subunit—the former being transcriptionally repressive—that translocates to the nucleus upon activation to regulate gene transcription.[13,15,16] In fact, angiotensin (Ang) II receptor antagonists confer renal protection apparently through inhibition of NF-κB-dependent proinflammatory pathways.[12,17] However, the putative factors triggering renal inflammation are not fully understood.

PTHrP displays proinflammatory features in various pathophysiological settings.[18] Recently, an increased interstitial macrophage influx was found to be associated with PTHrP overexpression in mice with folic acid-induced nephrotoxicity.[6] In this study, we explored whether PTHrP might be a key cytokine-inducing inflammation in the injured kidney.[19,20] We examined the functional consequences of chronic PTHrP upregulation in mouse kidney after unilateral ureteral obstruction (UUO), characterized by an early renal inflammatory response.[8,19–21] Furthermore, we assessed the putative intracellular pathways underlying the proinflammatory effects of PTHrP on renal tubuloepithelial cells.

RESULTS

Tubulointerstitial alterations occur associated with PTHrP upregulation in the obstructed mouse kidney

Endogenous mouse PTHrP mRNA levels increased similarly in the obstructed kidneys of control and PTHrP-overexpressing transgenic (PTHrP-TG) mice after UUO over corresponding levels in sham-operated mice (Figure 1a). By

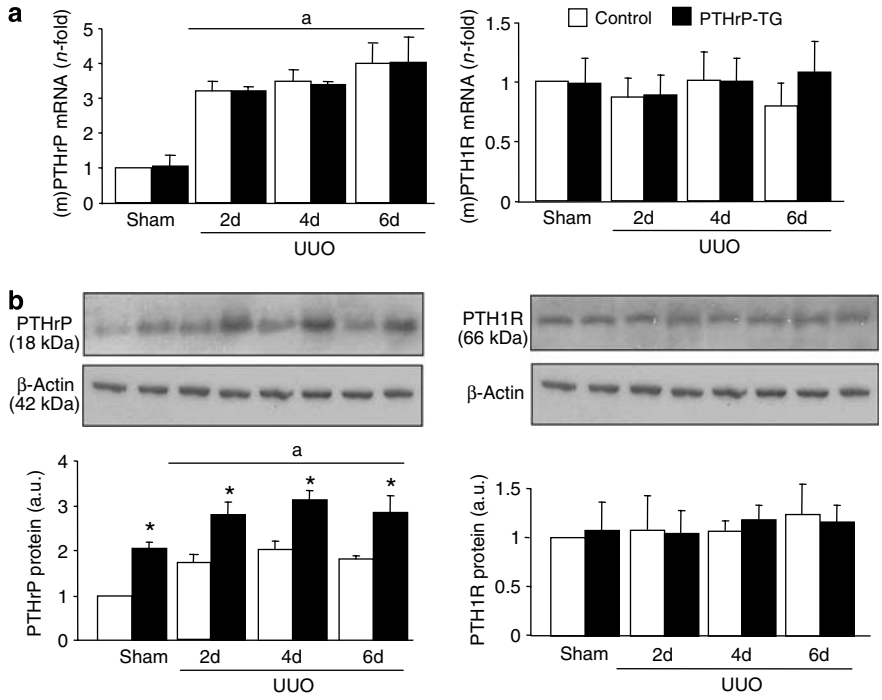

Figure 1 | PTHrP and PTH1R expression in the obstructed kidney from PTHrP-TG mice and their control littermates. In the kidney of sham-operated mice or the obstructed kidney for 2–6 days, we analyzed endogenous mouse (m)PTHrP gene expression (by real-time PCR) (**a**); PTHrP protein (by western blot, using antibody C6 recognizing both endogenous PTHrP and the human PTHrP transgene) (**b**); and the PTH1R gene (by real-time PCR) and protein (by western blot) expression (**a**, **b**). Representative autoradiograms are also shown in (**b**). Experimental values are mean ± s.e.m. of 4–6 mice per group at each time period. All values were normalized against corresponding sham control. $^{*}P<0.01$ vs corresponding value in control mice; $^{a}P<0.01$ vs corresponding value in sham-operated mice. AU, arbitrary units.

using PTHrP antiserum C6, recognizing the conserved 107-111 epitope in intact PTHrP in mice and humans,[3,6,22] PTHrP levels were twofold higher in the unobstructed kidney of PTHrP-TG mice than in that of control littermates and likewise increased after UUO (Figure 1b). A single band of an apparent molecular weight of 18 kDa was detected with this antiserum, corresponding to the single full-length PTHrP isoform in mice, as reported previously.[3,6] Meanwhile, PTH1 receptor (PTH1R) mRNA and protein levels remained unchanged within the same time period in these mice (Figure 1a and b).

Tubular atrophy and interstitial fibrotic areas in the obstructed kidneys occurred earlier, and the former was higher throughout the study in PTHrP-TG mice than in control littermates (Figure 2a). Both sham-operated kidneys and contralateral kidneys (data not shown) from PTHrP-TG mice showed no structural alterations and had a similar scarce number of interstitial leukocytes as in control littermates (Figure 2a and b). Starting 2 days after obstruction, a dramatic increase of the latter occurred in both types of mice, mainly in PTHrP-TG mice (Figure 2b).

PTHrP overexpression in the obstructed mouse kidney is related to increased proinflammatory factors

Various proinflammatory and chemotactic cytokines and cell adhesion molecules are involved in the inflammatory response after UUO.[8,14,19,21,23] In the unobstructed kidneys, we observed a higher (mRNA and/or protein) expression of monocyte chemoattractant protein (MCP)-1, MCP-1 receptor CCR2 (chemokine (C–C motif) receptor 2), RANTES (regulated upon activation, normal T-cell expressed, and secreted), and interleukin (IL)-6 in PTHrP-TG mice than in control mice (Figure 3). After UUO, there was a stepwise increase of the three former factors in the obstructed kidneys of control mice. This increase of MCP-1 protein and RANTES gene expression was higher in PTHrP-TG mice throughout the study (Figure 3a–c). Meanwhile, CCR2 gene expression reached its maximum earlier in the PTHrP-TG mice than in control littermates following obstruction (Figure 3a–c). A sustained increase of intercellular adhesion molecule (ICAM)-1 mRNA levels also occurred in the obstructed kidneys of the former animals (Figure 3d). In contrast, an early and transient increase of IL-6 gene expression was observed in these kidneys, which was more dramatic in PTHrP-TG mice (Figure 3e). Moreover, consistent with these findings and previous data,[14,19] we found elevated levels of NF-κB activity in the obstructed mouse kidney extracts, which were significantly higher in PTHrP-TG mice (Figure 4).

Effects of pharmacological suppression of PTHrP on inflammation in the obstructed mouse kidney

To confirm that PTHrP was responsible for the proinflammatory changes in the obstructed mouse kidney, we used several experimental maneuvers. First, PTHrP-TG mice were administered doxycycline (20 mg l^{-1} in drinking water;