Inhibitory effect of interleukin-1β on angiotensin II-induced connective tissue growth factor and type IV collagen production in cultured mesangial cells

Elsa Sánchez-López, Juan Rodríguez-Vita, Cecile Cartier, Monica Rupérez, Vanesa Esteban, Gisselle Carvajal, Raquel Rodríguez-Díez, Juan José Plaza, Jesús Egido, and Marta Ruiz-Ortega

1Cellular Biology in Renal Diseases Laboratory and 2Renal Unit, Fundación Jiménez Díaz, Universidad Autónoma Madrid, Madrid, Spain

Submitted 19 March 2007; accepted in final form 26 October 2007

Sánchez-López E, Rodríguez-Vita J, Cartier C, Rupérez M, Esteban V, Carvajal G, Rodríguez-Díez R, Plaza JJ, Egido J, Ruiz-Ortega M. Inhibitory effect of interleukin-1β on angiotensin II-induced connective tissue growth factor and type IV collagen production in cultured mesangial cells. Am J Physiol Renal Physiol 294: F149–F160, 2008. First published November 7, 2007; doi:10.1152/ajprenal.00129.2007.—Connective tissue growth factor (CTGF) is overexpressed in kidney diseases associated with extracellular matrix accumulation. Angiotensin II (ANG II) participates in renal fibrosis by the upregulation of growth factors, including CTGF, and extracellular matrix proteins, such as type IV collagen. During renal injury, ANG II and the macrophage-produced cytokine interleukin-1β (IL-1β) may be present simultaneously in the glomerular environment. However, there are no studies about the interaction between ANG II and IL-1β in renal fibrosis. For this reason, in cultured mesangial cells (MC), we investigated whether IL-1β could regulate ANG II-mediated collagen accumulation and the mechanisms underlying this process. In MC, CTGF is a downstream mediator of type IV collagen production induced by ANG II. IL-1β did not increase the production of CTGF and type IV collagen but significantly inhibited ANG II-induced CTGF and type IV collagen overexpression. Moreover, IL-1β also inhibited type IV collagen upregulation caused by exogenous recombinant CTGF. Matrix metalloproteinase-9 (MMP-9) is the main enzyme involved in type IV collagen degradation. In MC, coinubation of IL-1β and ANG II caused a synergistic increase in MMP-9 gene expression and activity, associated with type IV collagen inhibition. The described IL-1β effects were dependent on activation of ERK/MAPK but independent p38-MAPK, JNK, phosphatidylinositol 3-kinase/Akt, and Rho-associated kinase pathways. In summary, these data indicate that IL-1β-dependent on activation of protein kinases, for example, MAPK cascade, Rho-kinase, and include antagonistic activities of proinflammatory cytokines on ANG II actions.

transforming growth factor-β; extracellular-regulated kinase; metalloproteinases

MANY STUDIES HAVE DEMONSTRATED activation of the renal renin angiotensin system and increased local angiotensin II (ANG II) production in human and experimental kidney diseases. Blockade of ANG II, by angiotensin-converting enzyme inhibitors or AT1 antagonists, is one of the best options to treat renal diseases (35, 43). ANG II plays an important role in the regulation of kidney functions and participates in the progression of renal damage through the modulation of cell growth, fibrosis, and inflammation (23, 35, 37). These responses are mediated by the endogenous production of growth factors and cytokines (23, 33–35, 37). ANG II contributes to fibrosis by promoting extracellular matrix (ECM) protein production, through endogenous synthesis of transforming growth factor-β (TGF-β) and connective tissue growth factor (CTGF) (23, 33–35, 37).

The mechanisms of progressive glomerulonephritis remain mostly unknown. During renal injury, ANG II and proinflammatory cytokines, such as interleukin-1β (IL-1β), released by infiltrating inflammatory cells and intrinsic mesangial cells (MC), may be present simultaneously in the glomerular environment. These factors can activate MC to increase ECM production through the release of growth factors and therefore contribute to renal damage progression. However, data about the interrelation between the proinflammatory cytokine IL-1β and ANG II on MC behavior are scarce. IL-1β is an important cytokine with a broad range of biological activities (7) involved in kidney injury and repair (39, 42). In MC, IL-1β regulates cell growth, inflammation, and ECM proteins (6, 17, 28, 30), although there are no data about CTGF regulation.

Our aim was to investigate the effect of IL-1β in ANG II responses, evaluating in MC the effects on ECM regulation, as well as some potential molecular mechanisms involved. ANG II binds to specific receptors (AT1 and AT2) to activate cellular responses. AT1 receptor mediates upregulation of growth factors and fibrosis (23, 37). The AT1 signaling mechanisms are similar to those activated by cytokines, such as IL-1β, and include activation of protein kinases, for example, MAPK cascade, Rho-associated kinase (ROCK), and phosphatidylinositol 3-kinase (PI3-kinase)/Akt pathway (7, 8, 13, 16, 35). These in vitro experiments may help to elucidate the mechanisms of renal damage perpetuation and could suggest novel therapeutic strategies for modulating the biology of renal injury.

MATERIALS AND METHODS

Cell culture studies. Rat MC were cultured from isolated glomeruli by several sieving techniques and differential centrifugations (32). Glomerular MC were characterized by phase-contrast microscopy, positive staining for desmin and vimentin, and negative staining for keratin and factor VIII antigen, excluding epithelial and endothelial contamination, respectively. Cells were trypsinized, counted, grown in RPMI 1640 medium (GIBCO, Grand Island, NY), supplemented with 100 U/ml penicillin, 100 μg/ml streptomycin, and 2 mM glutamine in the presence of 20% heat-inactivated FBS, and cultured at 37°C.

Address for reprint requests and other correspondence: M. Ruiz-Ortega, Renal Unit, Fundación Jiménez Díaz, Avenida Reyes Católicos 2, 28040 Madrid, Spain (e-mail: mruizo@fjd.es).

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Fig. 1. Effect of IL-1β on connective tissue growth factor (CTGF) regulation in mesangial cells (MC). MC were treated with 1 ng/ml IL-1β and/or 10^{-7} mol/l ANG II for different times. A: CTGF gene expression was evaluated by Northern blot. Data were normalized by GAPDH expression. Similar results were found by real-time PCR. Top: representative Northern blot experiment. Bottom: results (means ± SE) of 4 experiments. *P < 0.05 vs. control. #P < 0.05 vs. ANG II. B: cells were treated with 1 ng/ml IL-1β and/or 10^{-7} mol/l ANG II for 24 h. Total CTGF protein levels were measured by Western blot. A representative experiment of 6 is shown.
37°C in 5% CO2 atmosphere. At confluence, cells were made quiescent for 48 h in RPMI 1640 with 0.5% FBS medium, and then different studies were performed. The experiments were done with cultured MC at passage 0 or 1.

Reagents. All culture reagents were purchased from Gibco BRL (Paisley, Scotland, UK). ANG II was obtained from Bachem. Recombinant IL-1β was from Peprotech (London, UK). Human recombinant CTGF was from MBL International (Woburn, MA).

The inhibitors used were PD-98059 (ERK1/2 inhibitor), SB-203580 (p38-MAPK inhibitor), and SP-600125 (JNK1, JNK2, and JNK3 inhibitor), all from Stressgen Bioreagents (Victoria, BC, Canada); wortmannin (PI3-kinase/Akt inhibitor) and matrix metalloproteinase-9 (MMP-9) inhibitor I, both from Calbiochem; and fasudil and Y-27632 (ROCK inhibitors) from Tocris Cookson (Bristol, UK). Cells were pretreated with all inhibitors 1 h before the stimulus was added. None of the inhibitors was toxic at the doses used, and none had an effect on basal CTGF levels.

The oligodeoxynucleotides (ODNs) were synthesized by Metabion (Martinsried, Germany). Cells were preincubated for 30 min with ODN antisense CTGF (5'-TACTGGCCGGCGGTCAT-3'), which blocks endogenous CTGF production, and ODN sense CTGF (5'-ATGACCGCGCCGAGTA-3') as control.

Gene and protein studies. RNA was isolated by Trizol (Invitrogen). CTGF gene expression was evaluated by Northern blot (36). Real-time PCR reactions were performed on ABI Prism 7500 sequence-detection PCR system (Applied Biosystems, Foster City, CA), and cDNA was synthesized with 2 μg of total RNA, according to manufacturer’s protocol. Assay identifications are as follows: Rn00573960_g1 for CTGF, Rn01401018_m1 for type IV procollagen, Rn00579162_m1 for MMP-9, Rn00575588_m1 for tissue inhibitor of metalloproteinase-1 (TIMP-1), Rn01513693_m1 for thrombospondin-1, and Rn00572010_m1 for TGF-β. Data were normalized with GAPDH and 18S ribosomal RNA expression (assay identifications: Rn99999916_s1 and Hs99999901_m1, respectively).

Cells were homogenized in lysis buffer (170 mmol/l Tris-HCl, 22% glycerol, 2.2% SDS, 0.1 mmol/l PMSF, orthovanadate, and protease inhibitor cocktail) and then separated by SDS-PAGE. CTGF protein and the phosphorylation levels of ERK1/2, p38, JNK, and Akt were determined in total protein extracts by Western blot (37). Type IV collagen and MMP-2 were analyzed in cell-conditioned medium, and 50 μg of proteins (quantified by bicinchoninic acid method) were loaded in each lane. The loading controls used were tubulin (for total proteins), total protein levels (in phosphorylation studies), or red Ponceau staining (for soluble proteins). Antibodies employed were CTGF from Torrey Pines Biolabs (Houston, TX); phospho-JNK1/2 from Stressgen Bioreagents; MMP-2, phospho-ERK1/2, ERK1/2, phospho-p38, phospho-Akt, Akt, JNK1/2, and type IV collagen from Santa Cruz Biotechnology (Santa Cruz, CA); and peroxidase-conjugated secondary antibodies from Amersham. Type IV collagen production was also determined by ELISA (Exocell).

MMPS activity was measured by zymography (44). Supernatants were loaded in 7.5% SDS-PAGE containing 1 mg/ml gelatin under nonreducing conditions. Next, gels were first incubated in developing buffer, then with Coomassie blue, and finally with 20% methanol-10% acetic acid. Total protein synthesis was evaluated by [3H]leucine incorporation (31).

Immunofluorescence. To determine type IV collagen production, cells were trypsinized and seeded in 24-well plates over crystal coverslips. After a 24-h serum-starvation step, cells were pretreated with several inhibitors for 1 h and then cells were stimulated for increasing times (24–72 h) with ANG II and/or IL-1β. Cells were then fixed in Merckofix (Merck), treated with 0.1% Triton X-100, and incubated with primary antibody against type IV collagen. After cells were washed, they were incubated with FITC-conjugated secondary antibody, and nuclei were stained with 1 μg/ml propidium iodide. The absence of primary antibody was the negative control. Samples were mounted in Mowiol 40-88 (Sigma) and examined by a laser scanning confocal microscope (Leica).

**RESULTS**

IL-1β inhibits ANG II-induced CTGF gene expression and protein production in MC. We have previously demonstrated that in MC ANG II elicited a rapid and sustained CTGF mRNA upregulation and protein production (37); however, there are no studies about CTGF regulation by IL-1β. In MC, IL-1β caused a slight but not significant increase in CTGF mRNA expression after 3 h, which diminished to control levels at 18 h but did not increase CTGF protein expression (Fig. 1). Surprisingly, IL-1β was found to suppress ANG II-induced CTGF mRNA expression at all times studied, between 3 and 24 h. This effect was dose dependent, with a maximal response at 10 ng/ml IL-1β. CTGF production caused by ANG II was also significantly decreased by IL-1β (Fig. 1B). This effect was not due to a general inhibition of protein expression because tubulin or total protein levels were not modified (determined by red Ponceau staining and [3H]leucine incorporation, respectively, not shown).

IL-1β does not modify TGF-β expression caused by ANG II in MC. ANG II and TGF-β share some responses involved in matrix regulation (23). ANG II increases TGF-β mRNA expression, protein production, and activation of latent TGF-β, through a thrombospondin-1-mediated process (25). In MC, we have observed that IL-1β and ANG II induced TGF-β gene expression that was not modified when both stimuli were added together, at the times studied until 24 h (Fig. 2). We have found that coincubation of IL-1β and ANG II also upregulated thrombospondin-1 expression, showing no differences to each stimuli alone (Fig. 2). These data suggest a different regulation of CTGF and TGF-β by IL-1β.

IL-1β attenuates type IV collagen gene expression and protein release induced by ANG II in MC. ANG II contributes to ECM accumulation (23, 35, 37). Stimulation of resting MC

![Fig. 2. Coincubation of IL-1β and ANG II does not modify transforming growth factor-β (TGF-β) and thrombospondin-1 gene expression. Cells were costimulated with 1 ng/ml IL-1β and 10^{-7} mol/l ANG II for 24 h. Values are means ± SE of 4 experiments of real-time PCR. *P < 0.05 vs. control.](http://ajprenal.physiology.org/10.21030/AJP-Renal-Physiol.9252)
with ANG II increased type IV procollagen gene and protein levels, as expected (Fig. 3). IL-1β has also upregulated several ECM proteins (28, 39), but we found that it had no effect on type IV collagen gene and protein production. Moreover, IL-1β markedly inhibited ANG II-induced type IV collagen gene expression, as well as cell-associated and soluble protein levels (at all times studied) (Fig. 3), showing a regulation similar to that observed with CTGF.

The next set of experiments was designed to demonstrate the link between CTGF and type IV collagen synthesis. CTGF increases ECM proteins, including type IV collagen (14). In MC, treatment with human recombinant CTGF (10 ng/ml)

![Fig. 3. A: effect of simultaneous presence of IL-1β and ANG II on type IV collagen gene expression. Cells were costimulated with 1 ng/ml IL-1β and 10^{-7} mol/l ANG II for 18 and 24 h. Values are means ± SE of 6 experiments of real-time PCR. IL-1β diminishes ANG II-induced cell-associated and soluble type IV collagen. B: cells were costimulated with 1 ng/ml IL-1β and 10^{-7} mol/l ANG II for 72 h. Values are means ± SE of 3 experiments of type IV collagen production. *P < 0.05 vs. control. #P < 0.05 vs. ANG II. C: Representative Western blot of soluble and cell-associated type IV collagen of 3 Western blot experiments. Tubulin was used as loading control.](http://ajprenal.physiology.org/)

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increased type IV collagen deposition (Fig. 4A). The blockade of endogenous CTGF by ODN antisense CTGF, but not with control sense ODN, markedly diminished ANG II-induced type IV collagen mRNA overexpression (44% inhibition, $P < 0.05, n = 3$, real time PCR, not shown) and protein production (Fig. 4A). These data show that inhibition of CTGF has a key role in the regulation of type IV collagen by ANG II in MC. In these cells, IL-1β also inhibited type IV collagen accumulation.
caused by recombinant CTGF, indicating that IL-1\(\beta\) regulates CTGF at two levels, by inhibiting its synthesis and its cellular responses.

Previous studies have demonstrated that ANG II upregulates CTGF via AT\(_1\) receptors and activation of MAPK, PKC, and ROCK pathways (15, 18, 38), but there are no studies in MC. The involvement of MAPK cascade was evaluated with the use of specific inhibitors of the p38-MAPK (SB-203580), ERK1/2 (PD-98059), and JNK (SP-600125) pathways (9, 19). We found that only the p38-MAPK inhibition, but not ERK and JNK inhibition, diminished ANG II-induced CTGF production in MC (Fig. 4B). In fibroblasts, the inhibitors of ERK1/2-MAPK and JNK-MAPK, but not p38-MAPK, decreased ANG II-stimulated CTGF expression (22), showing a different regulation depending on the cell type. In addition, only p38 inhibition diminished type IV collagen gene expression induced by ANG II (Fig. 4B). All of these results suggest that CTGF is a downstream mediator of ANG II-induced type IV collagen production and that this regulation requires p38-MAPK activation in MC.

**IL-1\(\beta\) inhibits ANG II-induced CTGF and type IV collagen through ERK-dependent pathway.** Next, we investigated the molecular mechanisms involved in CTGF and type IV collagen inhibition caused by IL-1\(\beta\). IL-1\(\beta\) activates several intracellular pathways shared with ANG II, including MAPK, ROCK, and PI3-kinase/Akt pathways (7, 8, 13, 16, 35). First, we evaluated the potential role of several intracellular signals in the inhibitory effect of IL-1\(\beta\) on CTGF regulation, using a pharmacological approach. Only the ERK1/2 inhibitor PD-98059, but not the p38 or JNK inhibitors, significantly blocked IL-1\(\beta\) effect, at both the gene (Fig. 5A) and protein levels (Fig. 5, B and C). The two ROCK inhibitors (fasudil and Y-27632) did not attenuate the IL-1\(\beta\) effect. PI3-kinase inhibition by wortmannin and LY-294002 blocks ANG II-stimu-
lated hyperplasia in cultured rat cells (40). Wortmannin did not affect CTGF inhibition caused by IL-1β. These data suggest that the IL-1β inhibitory effect seems to be mediated by activation of ERK cascade.

We also evaluated whether the same intracellular signals were involved in type IV collagen downregulation by IL-1β. As shown in Fig. 6, only pretreatment with the ERK1/2 inhibitor PD-98059, but not with the other MAPK inhibitors or with the ROCK and PI3-kinase inhibitors, significantly restored type IV procollagen gene levels, showing a similar response to CTGF regulation.

These results suggest that in MC ANG II regulates CTGF and type IV collagen via activation of p38 MAPK, whereas ERK1/2 participates in the IL-1β inhibitory effect.

**IL-1β and ANG II coinocubation synergistically increases ERK1/2, but not p38-MAPK, JNK, ROCK, or Akt activation.** In MC, IL-1β and ANG II triggered phosphorylation of all three MAPKs, including ERK, p38, and c-JNK (10, 13, 16). We compared the effect of IL-1β and ANG II on ERK activation (Fig. 7, A and B). Significant activation of ERK was detected within 2 min of ANG II stimulation, showed by enhanced phosphorylation of ERK1/2, remaining elevated at 30 min, but diminished to control levels after 50 min. IL-1β presented a delayed response, increasing ERK phosphorylation after 30 min and remaining elevated after 50 min. Coincubation of IL-1β and ANG II markedly enhanced ERK activation after 30 min. We also evaluated the effect of long-term incubation on ERK phosphorylation, showing no activation after 24 h with any stimulus.

Furthermore, we studied the activation of p38, JNK, and PI3-kinase/Akt pathways caused by ANG II and IL-1β alone or in combination. In MC, both ANG II and IL-1β activate p38, JNK, and PI3-kinase/Akt pathways (Fig. 7C). However, only the ERK pathway shows a synergistic activation when both stimuli are added together. Moreover, p38, JNK, and PI3-kinase/Akt pathways do not participate in the IL-1β inhibitory effect on ANG II-induced CTGF and type IV collagen expression, as shown in Figs. 5 and 6. These data suggest that ANG II and IL-1β caused a synergistic activation of ERK1/2 pathway that turns into blockade of CTGF and type IV collagen expression and protein production.

**Synergy between IL-1β and ANG II on MMP-9 activity.** Deposition of mesangial ECM is regulated by the balance between synthesis and degradation, the latter being regulated by MMPs. By zymography, we compared the effect of ANG II and IL-1β in two different MMPs: MMP-9 (with specificity toward type IV and V collagen) and MMP-2 (involved in fibronectin and laminin degradation) (20). IL-1β, but not ANG II, markedly increased MMP-9 gene expression. Coincubation of ANG II and IL-1β caused a synergistic upregulation on MMP-9 mRNA levels (Fig. 8A). Only IL-1β treatment significantly increased MMP-9 activity, shown by one lytic band corresponding to the inactive proform, which was not detectable under control conditions or in ANG II-treated cells. Coincubation of ANG II and IL-1β acted synergistically to increase MMP-9 activity (Fig. 8B). In contrast, MMP-2 activity and protein levels were not modified by both stimuli alone or in combination (Fig. 8C). Finally, we determined TIMP-1 expression levels, the main inhibitor of MMP-9 (2). As found with MMP-9, only IL-1β upregulated TIMP-1 gene but was not significantly modified by the coinocubation with ANG II (Fig. 8D). The ratio of MMP-9 to TIMP-1 indirectly defines MMP-9 activity (3). When ANG II and IL-1β are coinocubated, the MMP-9-to-TIMP-1 ratio is more than 30-fold (not shown), showing a clear matrix-degrading environment. Moreover, our data showed a time correlation between MMP-9 upregulation and collagen type IV inhibition, suggesting that this protein is being degraded. To further determine the direct contribution of MMP-9 on type IV collagen inhibition caused by IL-1β, we used MMP-9 inhibitor I (21). MC were pretreated with MMP-9 inhibitor I before stimulation with ANG II and IL-1β for 72 h. Inhibition of MMP-9 restored type IV collagen levels, abrogating the IL-1β inhibitory effect on ANG II response (Fig. 8E and 4A), suggesting that upregulation of MMP-9 by IL-1β plays a key role in type IV collagen degradation.

**The synergistic effect on MMP-9 is mediated by ERK-dependent pathway.** In MC, IL-1β elicited high levels of MMP-9 regulated by MAPK (10). We further evaluated the role of these intracellular signaling systems in the synergistic activation of MMPs caused by coinocubation of IL-1β and ANG II. Pretreatment with the ERK inhibitor PD-98059 diminished the synergistic gene upregulation and activation of MMP-9 (Fig. 8, A and B). In contrast, the inhibitors of p38-MAPK, ROCK, and PI3-kinase/Akt had no effect on this process.

**DISCUSSION**

Renal damage is associated with overexpression of a large variety of growth factors and cytokines, which influence the progression of kidney diseases. ANG II actively participates in renal fibrosis through the overexpression of growth factors,
Fig. 7. Coincubation of IL-1β and ANG II markedly increases ERK activation in MC. Cells were coincubated with 1 ng/ml IL-1β and 10^(-7) mol/l ANG II for different times. A: representative Western blot of phospho-ERK (p-ERK1/2) and ERK1/2 (used as control). B: values are means ± SE of 4 experiments. C: effect of coincubation of IL-1β and ANG II in activation of several kinases (p38-MAPK, JNK, PI3-kinase/Akt). Left: values are means ± SE of 3 experiments. Right: representative Western blot. *P < 0.05 vs. control. #P < 0.05 vs. ANG II. †P < 0.05 vs. IL-1β.
such as CTGF and TGF-β, and ECM proteins (23). Upregulation of IL-1β has been described in human and experimental renal injury (39, 42). These data suggest that, during renal injury, ANG II and IL-1β may be present simultaneously in the glomerular environment. In this paper, we investigated the interaction between ANG II and IL-1β in renal fibrosis. Although the direct in vivo effect of IL-1β in the kidney has not been evaluated, several studies have shown that IL-1β actively participates in the early inflammatory response and that the blockade of IL-1β receptor exerts renal protective effects (39,
However, its role in chronic renal injury and its contribution to fibrosis is unclear. In anti-Thy-1 nephritis, IL-1β receptor antagonism reduced MC proliferation and glomerular macrophage accumulation, but it had relatively little effect on glomerular ECM deposition (39). In rats with 5/6 nephrectomy, only overexpression of CTGF and TGF-β, but not IL-1β or TNF-α, was associated with interstitial fibrosis (12). In vitro studies in renal cells have shown different responses depending on the cell type. In tubulointerstitial cells, IL-1β participates in mesenchymal transition, through TGF-β and ECM regulation (11, 41). We found that, in cultured MC, IL-1β increased TGF-β but did not induce CTGF or type IV collagen production, a major component of expanded ECM in glomerular diseases. Moreover, we confirmed that IL-1β markedly up-regulates MMP-9 activity (24). These in vitro observations suggest that IL-1β actively participates in ECM degradation, and it is reasonable to speculate that IL-1β contributes to the remodeling processes associated with the glomerular inflammatory process.

CTGF is upregulated in many fibrotic disorders, including human kidney diseases, associated with ECM accumulation (29). In cultured renal cells, the blockade of CTGF diminished ECM accumulation caused by factors involved in renal damage progression, such as ANG II, TGF-β, and high glucose, suggesting that CTGF inhibition could represent a potential therapeutic approach to renal fibrosis (37). We observed that in cultured MC an ODN antisense CTGF, which blocks endogenous CTGF synthesis, diminished ANG II-induced type IV collagen accumulation, indicating that CTGF is a key factor involved in its regulation. We demonstrated that in MC IL-1β can suppress ANG II-induced CTGF gene expression and production. In fibroblasts, TNF-α also suppresses TGF-β- and dexamethasone-induced CTGF mRNA (1, 5), showing that proinflammatory cytokines abrogate CTGF overexpression in different cell types. In MC, we also found that IL-1β diminished type IV collagen upregulation caused by ANG II and recombinant CTGF, indicating that IL-1β regulates CTGF at two levels: by inhibiting its synthesis and its cellular responses.

The accumulation of mesangial ECM is the result of an excessive ECM production and an inhibition of its degradation. In MC, we observed that the simultaneous presence of IL-1β and ANG II caused a synergistic effect on MMP-9 gene expression and activity, as well as an increase in the ratio of MMP-9 to TIMP-1, indicating a matrix-degrading environment. MMP-9 upregulation by coinubcation with IL-1β and ANG II is responsible for type IV collagen degradation, as shown by the time-course correlation of regulation of both proteins and by the effect of MMP-9 inhibitor on type IV collagen levels.

In normal wound healing, there is an elevated ECM synthesis attributable to activation of resident renal cells by cytokines and growth factors until healing is completed. When persistent activation remains uncontrolled, ECM deposition occurs, leading to pathological fibrosis. However, how the wound healing process is normally regulated or leads to fibrosis has not been completely elucidated. Our in vitro findings in MC show that IL-1β is a negative regulator of ANG II-mediated type IV collagen accumulation through CTGF suppression and by increasing collagen degradation via MMP-9 upregulation. Although the biological meaning of this in vitro finding in renal pathology is not resolved in the present paper, our data show a novel mechanism involved in mesangial ECM regulation that could be involved in maintenance of tissue homeostasis or in the regulation of ECM deposition. In this sense, IL-1β has been shown to participate in the end of the wound healing process by keratinocytes through the inhibition of CTGF (26).

ANG II and TGF-β share some responses involved in matrix regulation (23). Many experimental evidences have shown that TGF-β is a mediator of ANG II actions. However, the data presented here show a different regulatory effect by IL-1β. In MC, IL-1β did not modify ANG II-induced gene overexpression of TGF-β or thrombospondin-1, the main activator of latent TGF-β, although it inhibited CTGF and type IV collagen production. In contrast, IL-1β markedly increased fibronectin, TIMP-1, and TGF-β1 production caused by TGF-β1 (28). These data show a clearly different response between ANG II and TGF-β.

Among the multiple signaling pathways involved in renal damage, we found that ERK-MAPK seems to be a major component mediating the inhibitory effect of IL-1β on ANG II-described responses. We demonstrated that specific inhibitors of the classical ERK cascade, but not p38-MAPK, JNK, PI3-kinase/Akt, or ROCK pathways, reverse the IL-1β-mediated effects on CTGF, type IV collagen, and MMP-9 regulation. In contrast, p38 activation by ANG II is required for the production of CTGF and type IV collagen, as previously described for TGF-β (25). These data show the complexity of the intracellular pathways involved in the regulation of profibrotic mediators and ECM components. Several experimental models have shown that the pharmacological blockade of ERK or p38 prevents ANG II-induced renal damage (4). Future in vivo studies are needed to know whether the modulation of ERK or p38-MAPK pathways could be important targets for the treatment of renal fibrosis.

During renal damage, there is an upregulation of cytokines and growth factors, with elevated local concentrations depending on the renal structures and progression of the disease (7, 23, 39, 42). Our in vitro data show that, if in the glomerular mesangial milieu there is a simultaneous presence of IL-1β and ANG II, IL-1β antagonizes ANG II-induced profibrotic response. Our findings are based on cell culture results and do not necessarily reflect the conditions in the fibrotic tissue in vivo, where other factors could also participate in the

![Diagram](http://ajprenal.physiology.org)  
**Fig. 9.** Scheme of the proposed mechanisms of IL-1β action on ANG II-induced CTGF and type IV collagen overexpression in cultured MCs.
regulation of ECM. In pathological conditions, the balance between profibrotic and antifibrotic mediators would finally decide whether these cells take part in excessive deposition of ECM or in an active remodeling process. Only future studies in human renal patients that would evaluate renal levels of a large array of cytokines in correlation with disease progression will help us to explain why some glomerulonephritis cases progress to end-stage kidney disease.

In summary, in cultured MC, we observed that the proinflammatory cytokine IL-1β abrogates ANG II-induced CTGF production and exerts an inhibitory effect on ANG II-mediated ECM accumulation. This IL-1β inhibitory effect was mainly mediated by ERK/MAPK pathway (Fig. 9).

These observations could help us to gain knowledge regarding the mechanisms involved in the regulation of tissue homeostasis and ECM accumulation.

ACKNOWLEDGMENTS

We thank Dr. Juan Antonio Moreno-Gutierrez for help with statistical analysis and Alberto Puime, Mar González-García-Parreño, and Sandra López León for technical help.

GRANTS

This work has been supported by grants from Fondo de Investigación Sanitaria (PI0205513, PI020822, ISCIII-RETIC-RD06/0004), Ministerio de Educación y Ciencia (SAF 2005-03378), and Sociedad Española de Nefrología. E. Sánchez-López and J. Rodriguez-Vita are fellows of Fondo de Investigación Sanitaria (PI0205513, PI020822, ISCIII-RETIC-RD06/0004), Ministerio de Educación y Ciencia (SAF 2005-03378), and Sociedad Española de Nefrología. G. Carvajal is a fellow of Fundación Carolina and Fundación Ibagó Alvarez de Toledo.

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