

Renal angiotensin II up-regulation and myofibroblast activation in human membranous nephropathy

SERGIO A. MEZZANO, CLAUDIO A. AROS, ALEJANDRA DROGUETT, M. EUGENIA BURGOS, LEOPOLDO G. ARDILES, CLAUDIO A. FLORES, DANIEL CARPIO, CARLOS P. VÍO, MARTA RUIZ-ORTEGA, and JESÚS EGIDO

Division of Nephrology, School of Medicine, Universidad Austral, Valdivia, Chile; Department of Physiology, Pontificia Universidad Católica, Santiago, Chile; and Fundación Jiménez Díaz, Universidad Autónoma, Madrid, Spain

Renal angiotensin II up-regulation and myofibroblast activation in human membranous nephropathy.

Background. The molecular mechanisms of renal injury and fibrosis in proteinuric nephropathies are not completely elucidated but the renin-angiotensin system (RAS) is involved. Idiopathic membranous nephropathy (MN), a proteinuric disease, may progress to renal failure. Our aim was to investigate the localization of RAS components in MN and their correlation with profibrotic parameters and renal injury.

Methods. Renal biopsies from 20 patients with MN (11 with progressive disease) were studied for the expression of RAS components [angiotensin-converting enzyme (ACE) and angiotensin II (Ang II)] by immunohistochemistry. Transforming growth factor- β (TGF- β) and platelet-derived growth factor (PDGF)-BB were studied by *in situ* hybridization, and myofibroblast transdifferentiation by α -smooth muscle actin (α -SMA) staining.

Results. ACE immunostaining was elevated in tubular cells and appeared in interstitial cells colocalized in α -actin-positive cells in progressive disease. Elevated levels of Ang II were observed in tubules and infiltrating interstitial cells. TGF- β and PDGF mRNAs were up-regulated mainly in cortical tubular epithelial cells in progressive disease ($P < 0.01$) and correlated with the myofibroblast transdifferentiation ($r = 0.8$, $P < 0.01$ for TGF- β ; $r = 0.6$, $P < 0.01$ for PDGF). Moreover, in serial sections of progressive cases, the ACE and Ang II over-expression was associated with the tubular expression of these profibrogenic factors, and with the interstitial infiltration and myofibroblast activation.

Conclusion. Intrarenal RAS is selectively activated in progressive MN. De novo expression of ACE at sites of tubulointerstitial injury suggests that the *in situ* Ang II generation could participate in tubular TGF- β up-regulation, epithelial-myofibroblast transdifferentiation, and disease progression. These results suggest a novel role of Ang II in human tubulointerstitial injury.

In recent years there has been growing evidence that abnormal glomerular permeability to plasma proteins and tubular traffic of filtered proteins can elicit an in-

flammatory and fibrogenic response, mediated by diverse chemokines and fibrogenic cytokines, that causes tubulointerstitial injury and a progressive decline in renal function [1]. The molecular mechanisms of renal injury and fibrosis in chronic proteinuric nephropathies are not completely elucidated but the renin-angiotensin system (RAS) is involved and seems to play a key role in progression to fibrosis [2, 3]. Therapies directed to RAS inhibition, such as angiotensin-converting enzyme inhibitors (ACEI) and AT₁ blockers, effectively limited or fully prevented progression of renal disease in animal models and human nephropathies [4, 5].

Idiopathic membranous nephropathy (MN) is a typical progressive human proteinuric renal disease, especially in cases with persistent nephrotic range proteinuria. We recently demonstrated in human biopsy specimens from patients with progressive MN a tubular over-activation of transcription factors [nuclear factor kappa B (NF κ B) and activator protein-1 (AP-1)] and an over-expression of proinflammatory [monocyte chemoattractant protein-1 (MCP-1), regulated upon activation, normal T cell expressed and secreted (RANTES), osteopontin] and profibrogenic [transforming growth factor- β (TGF- β), platelet-derived growth factor (PDGF)-BB] cytokines associated to an interstitial accumulation of mononuclear cells and an increase in myofibroblastic activity [6, 7]. Monocytes/macrophages are attracted to sites of injury by chemokines and these same infiltrating cells will further release inflammatory chemokines and cytokines, leading to a vicious cycle perpetuating the pathways responsible for progressive tubulointerstitial injury and fibrosis [8].

Angiotensin II (Ang II) has a pivotal role in the changes in glomerular hemodynamics and permeability properties of the filtration barrier contributing to proteinuria; however, in addition to these effects, Ang II has been directly implicated in tubulointerstitial injury and fibrosis acting as a local growth factor and directly influencing tubulointerstitial changes [3, 9, 10]. Intrarenal activation

of RAS seems to play a key role in this process and it may be up-regulated by proteinuria, as it was demonstrated in a recently published study in an animal model of intense proteinuria and interstitial nephritis induced by protein overload [11]. Moreover, evidence has been provided that Ang II may induce activation of NF κ B in renal tubular cells and vascular smooth muscle cells [12, 13].

There are few studies that have examined the localization of RAS components in human chronic proteinuric nephropathies such as MN. Therefore, we examined the intrarenal localization of diverse components of RAS and explored their potential functional correlation, particularly with profibrogenic cytokine expression and myofibroblast transdifferentiation. Our working hypothesis is that elevated local RAS during MN could be associated to the tubulointerstitial fibrosis and, therefore, contribute to the progression of the disease.

METHODS

Human kidney MN specimens have been examined from renal tissue of patients, as described in previous communications [6, 7]. Kidney samples were obtained by percutaneous renal biopsy from patients undergoing diagnostic evaluation at the Division of Nephrology, Austral University, Valdivia, Chile. The renal biopsies from 20 patients with idiopathic MN were studied (11 with progressive MN and 9 with non-progressive MN). The classification of progressive MN was done based on the presence of severe nephrotic syndrome (persistent massive proteinuria >8 g/day for at least 6 months), interstitial cell infiltration, and decrease of renal function at the time of the biopsy or during the following months. The 9 non-progressive MN patients were also nephrotic (proteinuria >3 g/day for less than 6 months), but without interstitial cell infiltration and with stable renal function at the time of the biopsy and during the long-term follow-up. None of the patients were being treated with ACE inhibitors or AT₁ antagonist at the time of the biopsy, and none of them had renal vein thrombosis.

Human control kidney specimens ($N = 5$) were taken from normal portions of renal tissue from patients who underwent surgery because of localized renal tumors. As control groups we also used some samples from patients with minimal change disease (MCD).

Light microscopy, immunohistochemistry, and in situ hybridization

Paraffin-embedded sections were treated for each technique as described [6]. Morphology was evaluated by hematoxylin and eosin (H&E), periodic acid-Schiff (PAS), and silver methenamine staining. Masson staining was used to evaluate the presence of interstitial fibrosis.

For immunohistochemistry in paraffin-embedded tissue, the following primary antibodies were employed:

anti-Ang II (rabbit antiserum, IHC 7002; Peninsula Laboratories, San Carlos, CA, USA) used as described [14]; a rabbit polyclonal antibody we developed, and raised against a synthetic peptide corresponding to the last 20 amino acids of the human ACE intracellular carboxyl-terminal sequence (peptide 1258-1277, SLHRHSHGPO FGSEVELRHS) was used to localize immunoreactive ACE as previously described [15, 16]. The antibody is directed against an intracellular domain of the enzyme and therefore recognizes the enzyme in its native, membrane-bound form at the site of synthesis, and does not cross-react with the plasma (circulating) enzyme, which is truncated in its region during its release into the circulation [15, 16]; specific biotinylated secondary antibodies, followed by streptavidin-horseradish peroxidase (HRP) conjugate (Dako Corp. Carpinteria, CA, USA), and revealed with diaminobenzidine (DAB), were used. For all staining, controls consisted of replacing the primary antibody with a nonspecific immunoglobulin G (IgG) of the same species.

Cryostat sections (5 μ m thick) from tissue fragments, frozen and kept in liquid nitrogen, were also used for the detection of T lymphocytes CD4 (mouse monoclonal, MT310; Dako Corp.) and T lymphocytes CD8 (mouse monoclonal, DK25; Dako Corp.).

In situ hybridization (ISH) was performed as described previously [6, 7]. Biotin-labeled human TGF- β and PDGF probes were purchased from R&D Systems (Minneapolis, MN, USA). The specificity of the reaction was confirmed by: (1) demonstrating the disappearance of hybridization signal when RNase (100 μ g/mL) (Sigma Chemical Co., St. Louis, MO, USA) was added in 0.05 mol/L Tris after the digestion with proteinase K; (2) the use of a sense probe (R&D Systems); (3) using a negative control (Plasmid DNA) (Dako Corp.); and (4) not using a probe.

Immunohistochemistry coupled with ISH: This technique was performed to simultaneously detect α -SMA-positive cells by IHC and cells expressing TGF- β by ISH on the same biopsy section, as described previously [6].

Histochemistry quantification

The percentage and intensity of the labeled surface area was evaluated by using a KS 300 Imaging System 3.0 (Zeiss). The degree of staining was calculated by the ratio of suitable binary threshold image and the total field area, integrating the intensity of the staining in the specific areas. Readings performed in this way allowed potential differences in the amount of total tissue examined to be avoided. For each sample, a mean value was obtained by analysis of 20 different fields (40 \times), excluding glomeruli and vessels. Quantification was done twice in a blinded manner and the interassay variations were not significant. The staining score is expressed as density/mm². This form of analysis, used to quantify immunohistochemical assays, has been validated in previous publications by our group [7].

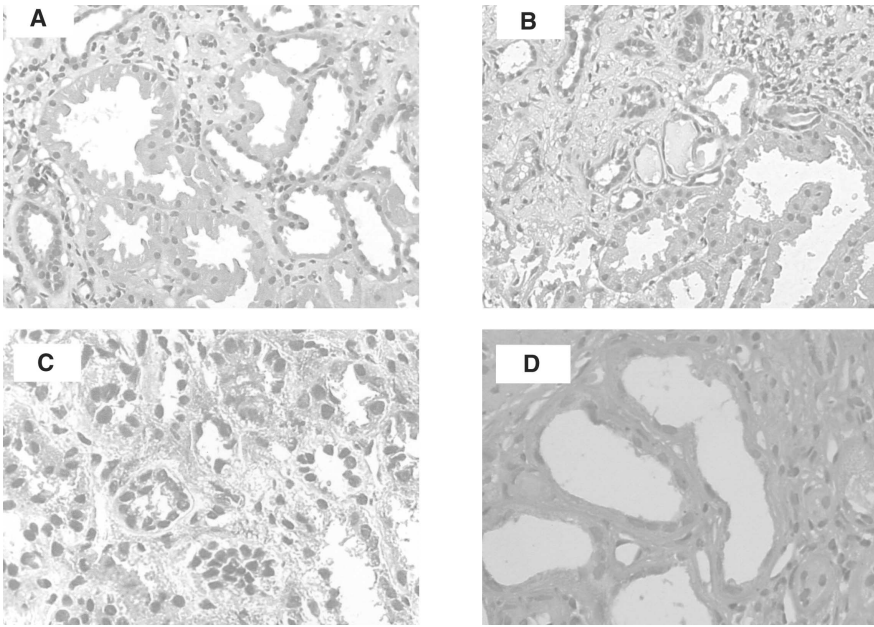


Fig. 1. Expression of components of the renin-angiotensin system (RAS) in idiopathic membranous nephropathy (MN). Magnification $\times 400$. Figure shows serial sections of two representative cases. (A) In contrast to that observed in normal kidneys, in which angiotensin-converting enzyme (ACE) was weakly expressed in the brush border of proximal tubules, a marked up-regulation was noted in some tubules, and (B, C) ACE neoinduction appeared in interstitial cells, mainly α -actin-positive cells. (D) Angiotensin II (Ang II) immunostaining was detected in tubular and interstitial infiltrating cells.

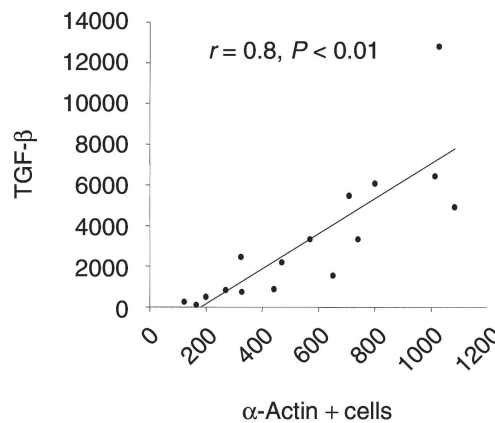
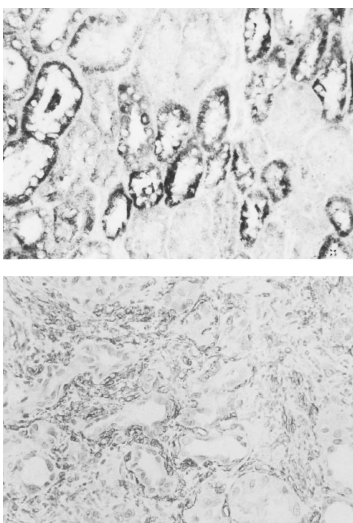


Fig. 2. Spearman correlation between the expression of transforming growth factor- β (TGF- β) mRNA by in situ hybridization, and α -smooth muscle actin by immunohistochemistry in progressive membranous nephropathy (MN). Staining score is expressed as density/ mm^2 . Magnification $\times 400$. Strong up-regulation of tubular TGF- β mRNA and interstitial myofibroblastic activity in progressive MN. Immunohistochemical staining for α -smooth muscle actin denotes interstitial myofibroblasts. The different techniques were done as described in **Methods**. Figure shows serial sections of a representative case. $r = 0.8, P < 0.01$.

Interstitial cell infiltration and fibrosis were classified into 4 groups according to their extent and the presence of tubular atrophy and degeneration: normal; involvement up to 25%; 26 to 50%; and extensive damage involving more than 50% of the cortex. Interstitial fibrosis was defined by the presence of interstitial collagen determined by Masson technique. Staining for α -smooth muscle actin (α -SMA) was particularly prominent in areas of fibrosis around atrophic tubules, defining vascular smooth muscle cells, activated mesangial cells, and interstitial myofibroblasts.

Statistical analysis

Statistical analyses were performed with the GraphPad Instat and GraphPad Software (San Diego, CA, USA).

Results of the clinical data are expressed as mean \pm SD. The intensity score and distribution of staining in the different techniques employed (IHC and ISH) are expressed as the mean \pm SEM. Mann-Whitney test was used to compare unpaired groups, Spearman’s correlation was used for the tubular TGF- β and PDGF mRNAs with the interstitial α -SMA over-expression. Fisher’s test was used when appropriate. A *P* value of <0.05 was considered significant.

RESULTS

Local RAS is activated in MN

In normal kidneys, ACE immunostaining was only found in the brush border membrane of tubular cells. In

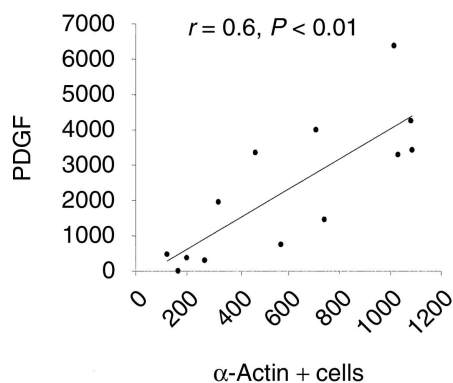
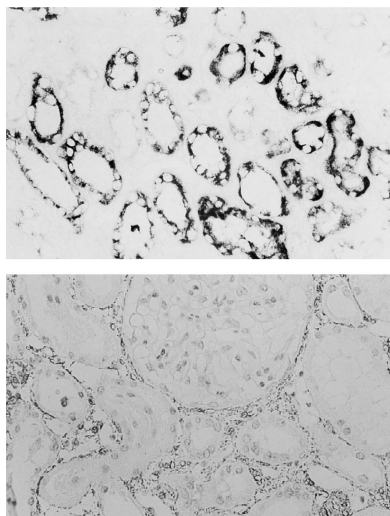


Fig. 3. Spearman correlation between the expression of platelet-derived growth factor (PDGF)-BB mRNA by in situ hybridization, and α -smooth muscle actin by immunohistochemistry in progressive membranous nephropathy (MN). Staining score is expressed as density/mm². Magnification $\times 400$. Strong up-regulation of tubular PDGF mRNA and interstitial myofibroblastic activity in progressive MN. Immunohistochemical staining for α -smooth muscle actin denotes interstitial myofibroblasts. The different techniques were performed as described in **Methods**. Figures show serial sections of a representative case. $r = 0.6$, $P < 0.01$.

samples from MN patients, a marked increase in ACE staining in this region was observed (Fig. 1A). In addition, a positive staining for ACE appeared in interstitial cells (Fig. 1B), mainly in α -actin-positive cells in sections of patients with progressive disease (Fig. 1C). We further determined whether local Ang II production was observed in MN. In the normal kidney, Ang II immunostaining was not expressed, while in MN there was a remarkable increase in Ang II production in tubular and interstitial infiltrating cells, mainly in progressive patients, as observed in Figure 1D.

Profibrogenic cytokines (TGF- β and PDGF-BB) are up-regulated in MN by ISH

In kidneys of MN patients, mRNA expression of the TGF- β was up-regulated mainly in cortical tubular epithelial cells (Fig. 2), and this expression was significantly higher in the group with progressive disease (mRNA TGF- β staining score was 5425 ± 1244 in progressive vs. 800 ± 295 in non-progressive MN, $P < 0.01$). No mRNA expression was found in control kidneys and in MCD. In general, the TGF- β mRNA expression was increased in the tubulointerstitial area, in a manner consistent with the severity of the disease, and particularly in tubules surrounded by α -actin-positive cells, as observed in the IHC coupled with ISH. At the glomerular level, we found a weak expression of this growth factor in some patients.

Platelet-derived growth factor mRNA expression was consistent with the expression of TGF- β and was observed mainly in cortical tubular epithelial cells, and primarily in cases with progressive disease (9 out of 11 vs. 3 out of 9, $P < 0.01$; mRNA staining score, 3350 ± 611 vs. 483 ± 310) (Fig. 3).

Interstitial myofibroblast infiltration in MN

α -Smooth muscle actin interstitial positive cells were mainly detected in patients with a progressive disease (11 out of 11 progressive vs. 3 out of 9 non-progressive, $P < 0.01$; staining score, 886 ± 105 vs. 208 ± 55) (Figs. 2 and 3). The staining distribution was mainly in the peritubular and periglomerular cortical interstitium, and there was a stronger expression in patients with progressive disease. The presence of myofibroblasts was strongly correlated with tubular over-expression of TGF- β ($r = 0.8$, $P < 0.01$) and PDGF ($r = 0.6$, $P < 0.01$), as shown in Figures 2 and 3. Moreover, the presence of myofibroblasts was significantly associated with the tubular interstitial cell infiltration and interstitial fibrosis ($P < 0.05$). In a double IHC to α -SMA coupled with ISH for TGF- β performed in some progressive cases, the immunostaining for α -SMA was observed mainly in the peritubular interstitium and in some tubular cells, and TGF- β mRNA expression localized in the surrounding tubular epithelial cells (Fig. 4).

Furthermore, there was a clear co-localization of interstitial ACE-positive cells, and myofibroblasts, as is shown in Figure 1C. Isolated glomerular α -actin-positive cells were observed and not associated with a higher incidence of glomerulosclerosis. In normal kidneys and sections of MCD, positive staining for α -actin only was observed constitutively in afferent arteriolar walls.

Correlation of renal RAS components and profibrogenic parameters in MN

In serial sections of different cases of progressive MN, the ACE over-expression and neoinduction were positively correlated with the tubular expression of TGF- β and PDGF, as well as with the interstitial infiltration and myofibroblast activation.

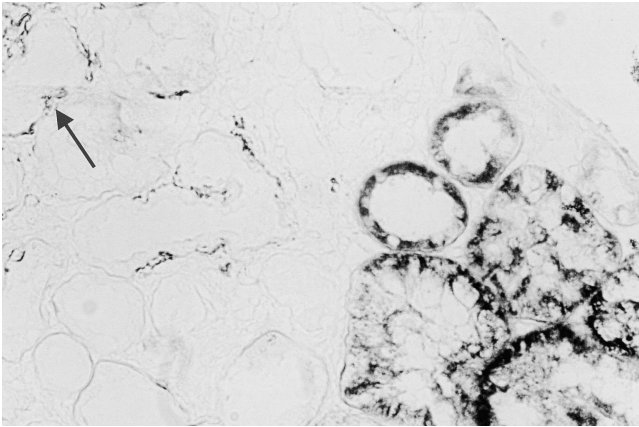


Fig. 4. Tubular epithelial myofibroblast transdifferentiation in progressive membranous nephropathy (MN). Immunohistochemistry for α -actin, coupled with in situ hybridization for transforming growth factor- β (TGF- β) resulted in a marked increase in TGF- β 1 tubular expression in areas with interstitial fibrosis, including tubules with phenotypic changes expressing α -smooth muscle actin (α -SMA) associated with disruption of the tubular basement membrane.

DISCUSSION

The tubulointerstitial injury is a major feature of MN and an important predictor of renal dysfunction. Our study reveals that renal biopsies from patients with MN present elevated levels of RAS components (increased ACE and Ang II expression in tubular and interstitial cells). Our current data also show a strong increase of tubuloepithelial cells expressing TGF- β and PDGF-BB mRNAs, which significantly correlates with the degree of tubulointerstitial damage and with the presence of interstitial myofibroblasts (α -SMA-positive cells). These parameters were correlated with the magnitude of proteinuria and the interstitial cell infiltration, particularly observed in patients with progressive membranous nephropathy.

The increased trafficking of proteins through the tubular cells can activate NF κ B and AP-1 and augment the tubular expression of target genes associated with interstitial cell infiltration and tubulointerstitial damage [7]. In addition, proteinuria may have a role in the intrarenal activation of RAS, as it was observed in an animal model of intense proteinuria and interstitial nephritis induced by protein overload [11]. In this study, a significant increase in angiotensinogen and ACE mainly localized in proximal and distal tubules and in the glomeruli was observed [11]. In our study, we observed an increase in tubular ACE and Ang II expression compared with controls; however, ACE neoinduction was also observed in renal myofibroblasts, and Ang II was localized in the interstitial inflammatory cells, thus contributing to the perpetuation of damage (Fig. 5). In a model of fibrosing kidney, interstitial fibroblast-like cells express RAS components [17], and production of Ang II by interstitial

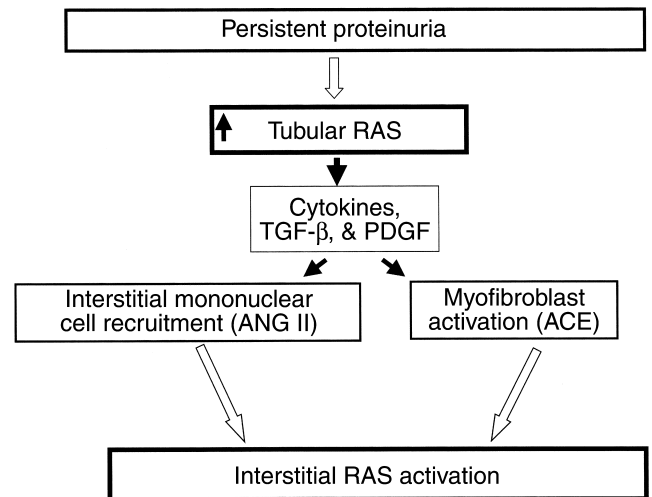


Fig. 5. In patients with membranous nephropathy (MN), there was an angiotensin-converting enzyme (ACE) and angiotensin II (Ang II) switched expression from tubular epithelial cells to interstitial cells coinciding with an activation of myofibroblasts, and recruitment of interstitial mononuclear cells, suggesting an additional novel participation of Ang II as a mediator of tubulointerstitial injury.

immunocompetent cells has also been described in experimental models of salt-sensitive hypertension [14], supporting our observations in human pathology of MN.

The finding of important tubulointerstitial lesions in nephropathies with persistent proteinuria suggests that mediators generated in tubular cells could be involved in this process. Locally generated Ang II could be secreted to the interstitial space, inducing the vasoconstriction of peritubular vessels with subsequent ischemia. In addition, Ang II could activate renal interstitial fibroblasts, inducing several growth-related metabolic events mediated by the AT₁ receptor [10]. Furthermore, there is ample evidence in vivo and in vitro that Ang II activates NF κ B and up-regulates NF κ B-related genes [12, 13, 18].

The final step in renal disease progression is atrophy and fibrosis of renal parenchyma. Transforming growth factor- β 1 and PDGF are two of the main profibrogenic cytokines. Numerous studies have shown that Ang II stimulates the expression of TGF- β 1 gene and protein in the kidney, thereby establishing the link between Ang II and fibrosis [3, 19–21]. In renal diseases in which there is a local activation of the RAS, even in the absence of systemic or intraglomerular hypertension, TGF- β 1-induced fibrosis may be via an Ang II-dependent mechanism [9].

Supporting our findings, a recently published study in the remnant kidney model in rats, by using morphologic techniques, showed an association between tubular protein load, peritubular accumulation of macrophages and myofibroblasts, and TGF- β 1 expression [22]. Interestingly, the RAS inhibition with an ACEI prevented proteinuria, as well as the inflammatory and fibrogenic re-

action [22]. Complementary to these findings is the hypothesis that tubular epithelial cells can transdifferentiate to a mesenchymal phenotype, migrate to the interstitium, and behave as true fibroblasts [23]. A recently published study addressed this problem and presented evidence suggesting that, via transition to a mesenchymal phenotype, tubular epithelial cells can produce extracellular matrix proteins in human disease and directly participate in the fibrotic process [24]. Myofibroblasts, identified by the expression of α -SMA, are the main source of extracellular matrix (ECM), by participating in progressive tubulointerstitial fibrosis, and are the best prognostic indicator of disease progression in both human and experimental glomerulonephritis [6, 25].

Possible mechanisms involved in tubular epithelial-myofibroblast transdifferentiation have been reviewed very recently [26], and TGF- β and PDGF-BB are able to induce transformation of fibroblasts into myofibroblasts [27, 28], supporting the strong correlation observed in this study.

This hypothesis has been supported by evidence in the model of 5/6 nephrectomy in rats, in which the tubular epithelial cells expressed α -SMA associated with disruption of the tubular basement membrane and ultrastructural changes consistent with an epithelial myofibroblast transdifferentiation [29]. The observation in human glomerulonephritis that tubular epithelial-myofibroblast transdifferentiation occurs in association with up-regulation of TGF- β , destruction of tubular basement membrane, loss of epithelial marker cytokeratin, and de-novo expression of α -SMA, supports the wide process of tubular epithelial-myofibroblast transdifferentiation [30], as shown in Figure 4 in a case of progressive MN.

As we noted, TGF- β is a key regulator of the tubular epithelial-myofibroblast transdifferentiation (TEMT) in different tissues [24, 31, 32], and recent studies have shown that TGF- β regulates TEMT through its intracellular signaling pathway, Smad2 [33]. TGF- β -induced Smad2 phosphorylation results in the transformation of tubular epithelial cells into a myofibroblast phenotype with the loss of E-cadherin and de novo expression of α -SMA and collagens in normal rat tubular epithelial cells [34]. In addition, Smad signaling has been described as a novel pathway of Ang II-induced renal fibrosis by inducing TEMT mediated by the AT₁ receptor [35].

CONCLUSION

In patients with MN there was an ACE-switched expression from tubular epithelial cells to interstitial cells coinciding with an activation of myofibroblasts, suggesting an additional novel participation of Ang II as a mediator of tubulointerstitial injury.

ACKNOWLEDGMENTS

This work was supported by research grants from Fondo Nacional de Investigación Científica y Tecnológica, CHILE (FONDECYT 1000433, 1010373), Dirección de Investigación, Universidad Austral, Valdivia, and Ministerio de Ciencia y Tecnología (SAF 2001-0717), Fondo de Investigación Sanitaria (PI020513) España.

Reprints requests to Sergio Mezzano, M.D., Department of Nephrology, School of Medicine, Universidad Austral, P.O. Box 8-D, Valdivia, Chile.

E-mail: smezzano@uach.cl

REFERENCES

1. REMUZZI G, RUGGENENTI P, BEGNINI A: Understanding the nature of renal disease progression. *Kidney Int* 51:2–15, 1997
2. REMUZZI G, BERTANI T: Pathophysiology of progressive nephropathies. *N Eng J Med* 339:1448–1456, 1998
3. MEZZANO S, RUIZ-ORTEGA M, EGIDO J: Angiotensin II and renal fibrosis. *Hypertens* 38:635–638, 2001
4. THE GISEN GROUP: (Gruppo Italiano di Studi Epidemiologici in Nefrologia): Randomised placebo-controlled trial of effect of ramipril on decline in glomerular filtration rate and risk of terminal renal failure in proteinuric non diabetic nephropathy. *Lancet* 349: 1857–1863, 1997
5. GANSEVOORT RT, NAVIS GJ, WAPSTRA FH, et al: Proteinuria and progression of renal disease: Therapeutic implications. *Curr Opin Nephrol Hypertens* 6:133–140, 1997
6. MEZZANO SA, DROGUETT A, BURGOS ME, et al: Overexpression of chemokines, fibrogenic cytokines, and myofibroblasts in human membranous nephropathy. *Kidney Int* 57:147–158, 2000
7. MEZZANO SA, BARRÍA M, DROGUETT A, et al: Tubular NF- κ B and AP-1 activation in human proteinuric renal disease. *Kidney Int* 60:1366–1377, 2001
8. ABBATE M, ZOJA C, CORNA D, et al: In progressive nephropathies, overload of tubular cells with filtered proteins translates glomerular permeability dysfunction into cellular signals of interstitial inflammation. *J Am Soc Nephrol* 9:1213–1224, 1998
9. BORDER WA, NOBLE NA: Interactions of transforming growth factor- κ and angiotensin II in renal fibrosis. *Hypertens* 31:181–188, 1998
10. RUIZ-ORTEGA M, EGIDO J: Angiotensin II modulates cell-growth related events and synthesis of matrix proteins in renal interstitial fibroblasts. *Kidney Int* 52:1497–1510, 1997
11. LARGO R, GÓMEZ-GARRE D, SOTO K, et al: Angiotensin-converting enzyme is upregulated in the proximal tubules of rats with intense proteinuria. *Hypertens* 33:732–739, 1999
12. GÓMEZ-GARRE D, LARGO R, TEJERA N, et al: Activation of NF- κ B in tubular epithelial cells of rats with intense proteinuria. Role of angiotensin II and endothelin-1. *Hypertens* 37:1171–1178, 2001
13. RUIZ-ORTEGA M, LORENZO O, RUPEREZ M, et al: Angiotensin II activates nuclear transcription factor kappa B through AT(1) and AT(2) in vascular smooth muscle cells—Molecular mechanisms. *Circ Res* 86:1266–1272, 2000
14. RODRÍGUEZ-ITURBE B, PONS H, HERRERA-ACOSTA J, JOHNSON R: Role of immunocompetent cells in nonimmune renal diseases. *Kidney Int* 59:1626–1640, 2001
15. LOMBARDI DM, VISWANATHAN M, VÍO CP, et al: Renal and vascular injury induced by exogenous angiotensin II is AT1 receptor-dependent. *Nephron* 87:66–74, 2001
16. SUGA S-I, PHILLIPS MI, RAY PE, et al: Hypokalemia induces renal injury and alterations in vasoactive mediators that favor salt sensitivity. *Am J Physiol Renal Physiol* 281:F260–F269, 2001
17. OKADA H, INOUE T, KANNO Y, et al: Interstitial fibroblast-like cells express renin-angiotensin system components in a fibrosing murine kidney. *Am J Pathol* 160:765–772, 2002
18. RUIZ-ORTEGA M, LORENZO O, SUZUKI Y, et al: Proinflammatory actions of angiotensin II. *Curr Opin Nephrol Hypert* 10:321–329, 2001
19. WOLF G: Link between angiotensin II and TGF-beta in the kidney. *Miner Electrolyte Metab* 24:174–180, 1998

20. BORDER WA, NOBLE NA: Transforming growth factor β in tissue fibrosis. *N Engl J Med* 331:1286–1292, 1994
21. NOBLE NA, BORDER WA: Angiotensin II in renal fibrosis: Should TGF- β rather than blood pressure be the therapeutic target? *Semin Nephrol* 17:455–466, 1997
22. ABBATE M, ZOJA C, ROTTOLI D, et al: Proximal tubular cells promote fibrogenesis by TGF- β 1-mediated induction of peritubular myofibroblasts. *Kidney Int* 61:2066–2077, 2002
23. ZEISBERG M, STRUTZ F, MULLER GA: Renal fibrosis: An update. *Curr Opin Nephrol Hypertens* 10:315–320, 2001
24. RASTALDI MP, FERRARIO F, GIARDINO L, et al: Epithelial-mesenchymal transition of tubular epithelial cells in human renal biopsies. *Kidney Int* 62:137–146, 2002
25. ROBERTS IS, BURROWS C, SHANKS JH, et al: Interstitial myofibroblasts: Predictors of progression in membranous nephropathy. *J Clin Pathol* 50:123–127, 1997
26. LAN HY: Tubular epithelial-myofibroblast transdifferentiation mechanisms in proximal tubule cells. *Curr Opin Nephrol Hypert* 12:25–29, 2003
27. TANG WW, ULICH TR, LACEY DL, et al: Platelet-derived growth factor-BB induces renal tubulointerstitial myofibroblast formation and tubulointerstitial fibrosis. *Am J Pathol* 148:1169–1180, 1996
28. DESMOULIÈRE A, GEINOZ A, GABBIANI F: Transforming growth factor- β 1 induces α -smooth muscle actin expression in granulation tissue myofibroblasts and in quiescent and growing cultured fibroblasts. *J Cell Biol* 122:103–111, 1993
29. NG YY, HUANG TP, YANG WC, et al: Tubular epithelial-myofibroblast transdifferentiation in progressive tubulointerstitial fibrosis in 5/6 nephrectomized rats. *Kidney Int* 54:864–876, 1998
30. JINDE K, NIKOLIC-PATERSON DJ, HUANG XR, et al: Tubular phenotypic change in progressive tubulointerstitial fibrosis in human glomerulonephritis. *Am J Kidney Dis* 38:761–769, 2001
31. FAN JM, NG YY, HILL PA, et al: Transforming growth factor-beta regulates tubular epithelial-myofibroblast transdifferentiation in vitro. *Kidney Int* 56:1455–1467, 1999
32. FAN JM, HUANG XR, NG YY, et al: Interleukin-1 induces tubular epithelial-myofibroblast transdifferentiation through a transforming growth factor-beta1-dependent mechanism in vitro. *Am J Kidney Dis* 37:820–831, 2001
33. ZIMMERMAN CM, PADGETT RW: Transforming growth factor B signaling mediators and regulators. *Gene* 249:17–30, 2000
34. LI HJ, ZHU HJ, HUANG XR, et al: Smad7 inhibits fibrotic effect of TGF-beta on renal tubular epithelial cells by blocking Smad2 activation. *J Am Soc Nephrol* 13:1464–1472, 2002
35. HUANG XR, LI JH, CHEN YX, et al: SMAD signaling, a novel pathway of angiotensin II-induced renal fibrosis. *J Am Soc Nephrol* 12:465, 2001