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# Renin-angiotensin system activation and interstitial inflammation in human diabetic nephropathy

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**Renin-angiotensin system activation and interstitial inflammation in human diabetic nephropathy.**

*Background.* The molecular mechanisms of renal injury in diabetic nephropathy (DN) are not completely understood, although inflammatory cells play a key role. The renin-angiotensin system (RAS) is involved in kidney damage; however, few studies have examined the localization of RAS components in human DN. Our aim was to investigate in renal biopsies the expression of RAS and their correlation with proinflammatory parameters and renal injury.

*Methods.* The biopsies from 10 patients with type 2 diabetes mellitus and overt nephropathy were studied for the expression of RAS components by immunohistochemistry (IH). In addition, by Southwestern histochemistry we studied the in situ detection of the activated nuclear factor kappa B (NFκB), and by IH and/or in situ hybridization (ISH), the expression of monocyte chemoattractant protein-1 (MCP-1) and regulated upon activation, normal T cell expressed and secreted (RANTES), whose genes are regulated by NFκB.

*Results.* Angiotensin-converting enzyme (ACE) immunostaining was elevated in tubular cells and appeared in interstitial cells. Elevated levels of angiotensin II (Ang II) immunostaining were observed in tubular and infiltrating interstitial cells. There was also a down-regulation of $AT_1$ and up-regulation of $AT_2$ receptors. An activation of NFκB and a marked up-regulation of NFκB-dependent chemokines mainly in tubular cells was observed. Elevated levels of NFκB, chemokines, and Ang II in tubules were correlated with proteinuria and interstitial cell infiltration.

*Conclusions.* Our results show that in human DN, RAS components are modified in renal compartments, showing elevated local Ang II production, activation of tubular cells, and induction of proinflammatory parameters. These data suggest that Ang II contributes to the renal inflammatory process, and may explain the molecular mechanisms of the beneficial effect of RAS blockade.

Diabetic nephropathy (DN) is the most common human renal disease in the Western world, although its pathogenesis is not completely understood. Despite the fact that this condition is not considered an inflammatory disease, the presence of interstitial mononuclear cells is widely recognized. The nuclear factor kappa B (NFκB) is a transcription factor that regulates the gene expression of several proinflammatory mediators, including cytokines, adhesion molecules, and chemokines [1–3]. Among these genes, monocyte chemoattractant protein-1 (MCP-1) and regulated on activation normal T-cells expressed and secreted chemokine (RANTES) play important roles in the recruitment of inflammatory cells [3–5]. Activation of renal NFκB has been described in human and experimental nephritis [3, 6–8]. In human proteinuric nondiabetic renal disease, we have shown that tubular overactivation of NFκB, and simultaneous up-regulation of certain proinflammatory and profibrogenic genes, are markers of progressive renal disease [9]. The beneficial effects of NFκB inhibition have recently been tested in experimental renal immune diseases [10].

Activation of local renin-angiotensin system (RAS) seems to be involved in the progression of kidney damage, including DN. Studies in experimental animal models of DN have demonstrated up-regulation of RAS components and beneficial effect of RAS blockade [11]. In human DN, treatment with angiotensin-converting enzyme (ACE) inhibitors and $AT_1$ antagonists retarded disease progression. Results from large clinical trials have recently confirmed the beneficial effects of these drugs in DN [12–14]. Although some evidence suggests activation of intrarenal RAS in diabetes [11], the localization of renal expression of RAS components, as well as the source of intrarenal angiotensin II (Ang II) and its potential relation with inflammation, remains unclear. Ang II is a cytokine that participates in the inflammatory response in the kidney through the regulation of NFκB and related genes, such as cytokines, adhesion molecules, and the chemokines MCP-1 and RANTES [15, 16]. Our working hypothesis is that elevated intrarenal Ang II activity may contribute to the inflammatory response and thereby to the progression of kidney damage in DN.

## METHODS

### Human kidney specimens

Kidney samples were obtained by percutaneous renal biopsy from patients undergoing diagnostic evaluation at the Division of Nephrology, Austral University, Valdivia, Chile. Renal biopsies from 10 patients with type 2 diabetes mellitus and overt nephropathy were studied. None of the patients had good metabolic control. Four out of 10 patients had proteinuria in the nephrotic range. None of the patients were being treated with ACE inhibitors or $AT_1$ antagonists 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.

### Light microscopy, Southwestern histochemistry, in situ hybridization and immunohistochemistry

Paraffin-embedded sections were treated for each technique as previously described [9]. 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.

Southwestern histochemistry (SWH) was done as described earlier [9]. Controls included absence of probe, mutant NFκB DIG-labeled probe (sense: 5′-AGTTGA GGCTCCTTTCCCAGGC-3′), and competition assays with a 200-fold excess of unlabeled NFκB, followed by incubation with labeled probe. In situ hybridization (ISH) was performed as described previously [9, 17]. Biotin-labeled human MCP-1 and RANTES probes were purchased from R&D Systems (Minneapolis, MN, USA). The specificity of the reaction was confirmed: (*1*) by demonstrating the disappearance of hybridization signal when RNAse (100 ug/mL) (Sigma Chemical Co., St. Louis, MO, USA) was added to 0.05 mol/L Tris after the digestion with proteinase K; (*2*) with the use of a sense probe (R&D Systems); (*3*) with a negative control (Plasmid DNA) (Dako Corp., Carpinteria, CA, USA); and (*4*) without a probe. For immunohistochemistry (IMH) in paraffin-embedded tissue, the following primary antibodies were employed: anti-MCP-1 [goat polyclonal immunoglobulin G (IgG), AB-279-NA; R&D Systems)]; anti-RANTES (goat polyclonal, AB-278-NA; R&D Systems); anti-OPN (mouse monoclonal, MPIIIB10; Development Studies Hybridoma Bank, Iowa City, IA, USA); anti-NFκB p50 (goat polyclonal IgG, sc-1191; Santa Cruz Biotechnology, Santa Cruz, CA, USA); anti-NFκB p65 (mouse monoclonal, cat 1697838; Boehringer Mannheim, Mannheim, Germany); anti-Ang II (rabbit antiserum, IHC 7002; Peninsula Laboratories, San Carlos, CA, USA), used as described [18]; A rabbit polyclonal antibody (A28A9) developed by our group and raised against a synthetic peptide corresponding to the last 20 amino acids of the human ACE intracellular carboxyl-terminal sequence (peptides 1258–1277, SLHRH SHGPQFGSEVELRHS) was used to localize immunoreactive ACE. The specificity of the antisera has been well established in Western blots and immunohistochemistry [19]. 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 this region during its release into the circulation [19]. Furthermore, antibodies to $AT_1$ and $AT_2$ (rabbit polyclonal, Santa Cruz), CD68 (macrophage marker, Dako Corp.), and α-smooth muscle actin (α-SMA) were used. Specific biotinylated secondary antibodies, followed by streptavidin-horseradish-peroxidase (HRP) conjugate (Dako Corp.), and revealed with diaminobenzidine (DAB), were used. For all staining, controls consisted of replacing the primary antibody with a nonspecific IgG of the same species.

Cryostat sections (5 μm 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.).

### Histochemistry quantification

The percentage and intensity of the labeled surface area was evaluated by using a KS 300 Imaging System 3.0 (Zeiss, München-Hallbergmoos, Germany). 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. Performing the readings in this way allows 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 (×20), 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/$mm^2$. This form of analysis, used to quantify immunohistochemical assays, has been validated in previous publications by our group [9].

Interstitial cell infiltration and fibrosis were classified into four groups according to their extent and the presence of tubular atrophy and degeneration: (*1*) normal; (*2*) involvement up to 25%; (*3*) 26 to 50%; and (*4*) 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-SMA was particularly prominent in areas of fibrosis around atrophic tubules, defining vascular smooth muscle cells, activated mesangial cells, and interstitial myofibroblasts.

**Table 1.** Clinical data and staining score for NFκB of patients with diabetic nephropathy

| Case | Sex | Age | Serum creatinine *mg/dL* | Proteinuria *g/day* | MAP *mm Hg* | NFκB |
|---|---|---|---|---|---|---|
| 1 | F | 45 | 0.8 | <3.5 | 93 | 3361 |
| 2 | F | 40 | 1.6 | <3.5 | 110 | 4468 |
| 3 | F | 42 | 0.9 | >3.5 | 103 | 6080 |
| 4 | F | 51 | 2.3 | >3.5 | 120 | 9851 |
| 5 | M | 54 | 1.4 | >3.5 | 120 | 8694 |
| 6 | F | 55 | 1.4 | >3.5 | 123 | 12408 |
| 7 | M | 55 | 1.5 | <3.5 | 140 | 980 |
| 8 | F | 56 | 1.7 | <3.5 | 133 | 14153 |
| 9 | F | 60 | 2.1 | <3.5 | 120 | 4676 |
| 10 | F | 62 | 2.0 | <3.5 | 115 | 4493 |
| Mean | | 52 ± 7 | 1.57 ± 0.48 | | 118 ± 14 | 6916 ± 1332 |

Abbreviations are: MAP, mean arterial pressure; NFκB, nuclear factor kappa B. Staining score is expressed as density/mm$^2$.

### 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 ± SD. The intensity score and distribution of staining in the different techniques employed (SWH, IMH, and ISH) are expressed as the mean ± SEM. Spearman's correlation was used to correlate the tubular NFκB activation with the tubular MCP-1 and RANTES over-expression. Fisher's test was used when appropriate. A $P$ value of $<0.05$ was considered significant.

## RESULTS

### Clinical data

Table 1 summarizes the clinical data for each patient studied and the staining score of renal NFκB activation by SWH. Eight out of 10 patients were female, with a mean age of 52 ± 7 years. Renal function deterioration was present in five out of 10 patients, with a serum creatinine over 1.5 mg/dL, and four patients (cases 3 to 6) had nephrotic syndrome with proteinuria over 3.5 g/day. A mean arterial pressure (MAP) over 110 mmHg was present in eight out of 10 patients.

### Local RAS is activated in DN

In normal kidneys, ACE immunostaining was only found in the brush border membrane of tubular cells. In samples from DN patients, a marked increase in ACE staining in this region was observed. In addition, a positive staining for ACE appeared in interstitial cells, mainly in α-actin positive cells (Fig. 1). We further determined whether local Ang II production was observed in DN. In the normal kidney, Ang II immunostaining was weakly expressed, while in DN there was a remarkable increase in Ang II production in tubular and interstitial infiltrating cells (Fig. 1). $AT_1$ receptor expression was lower (423 ± 82 density/mm$^2$) than $AT_2$ receptor expression (1061 ± 221 density/ mm$^2$) in the diabetic kidneys.

### NFκB is activated in patients with DN

Activated NFκB was observed in the kidneys of all patients studied (Table 1), in comparison with the absence in normal control kidneys (not shown). There was a significant association between the magnitude of proteinuria and the staining score for NFκB (mean, 6916 ± 1332 density/ mm$^2$; $P < 0.05$). Patients with proteinuria >3.5 g/day had significantly higher ($P < 0.05$) NFκB staining score (9258 ± 1313) than patients with proteinuria <3.5 g/day (5355 ± 1848) . Active NFκB complexes were mainly detected in cortical tubular epithelial cells and, in a sparse manner, in some glomerular cells, mainly podocytes (Fig. 2A). Some nuclei of parietal and endothelial cells were also positive for activated NFκB. This transcription factor consists of a heterodimer of p65 and p50 subunits [1] that were mainly detected in tubular epithelial cells (cytoplasm and nuclei), without significant differences between the samples studied (not shown).

### Proinflammatory chemokines are up-regulated in DN

In kidneys of DN patients, mRNA expression of the chemokines MCP-1 (mean, 19,401 ± 4318 density/mm$^2$) and RANTES (mean, 11,914 ± 3063 density/mm$^2$) was mainly detected in tubular epithelial cells (Fig. 2 D and F), while no mRNA expression was found in control kidneys. Similar results were noted in the protein expression of these chemokines (MCP-1 mean, 1628 ± 236; RANTES, 1215 ± 268 density/mm$^2$), with a major immunostaining in kidneys with progressive disease, and with a pattern similar to that observed for their respective mRNAs (Fig. 2). A marked correlation was found between gene and protein expression and localization of both chemokines in the same tubular cells. In addition, in some DN patients, mild glomerular staining for MCP-1 and RANTES was noted both at mRNA and protein levels. Moreover, we studied the expression of osteopontin protein (OPN), and this chemokine was mainly detected in tubular epithelial cells of DN patients, with a similar pattern to that of MCP-1 and RANTES (Fig. 2B).

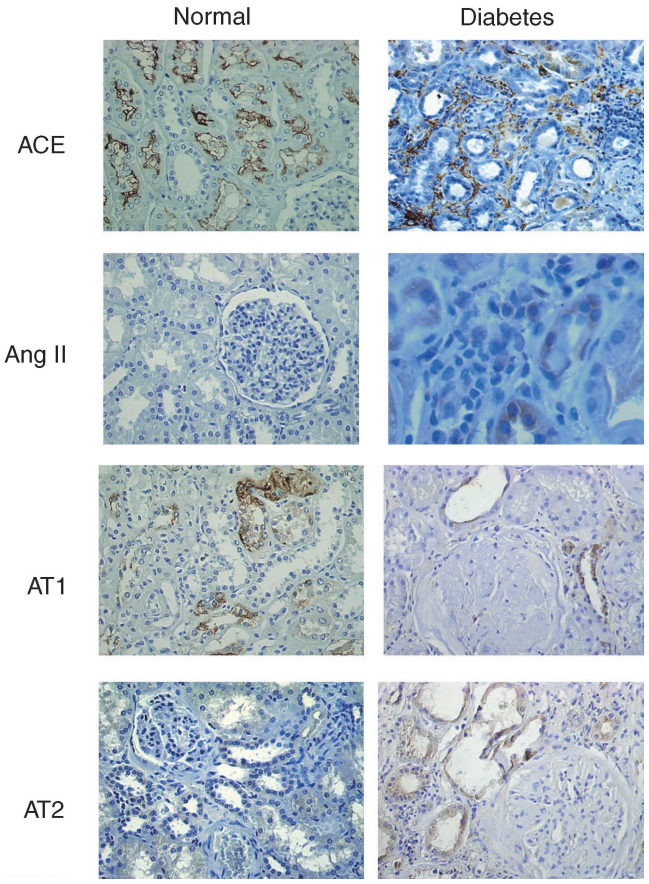


**Fig. 1. Expression of components of the renin-angiotensin system.** Shown are serial sections of one representative case of diabetes, and one normal kidney section. In contrast to that observed in the normal kidney, in which angiotensin-converting enzyme (ACE) was weakly expressed in the brush border of proximal tubules, a marked up-regulation was noted in some tubules, and ACE neoinduction appeared in interstitial cells, mainly α-actin positive cells. Angiotensin II (Ang II) immunostaining was detected in tubular and interstitial infiltrating cells. In comparison with the widespread distribution of $AT_1$ and $AT_2$ receptors in normal kidney, in diabetic nephropathy (DN) the staining score for $AT_1$ was significantly decreased as compared with the $AT_2$ receptor. Magnification ×200 and ×400 normal kidney vs. diabetic kidney, respectively.

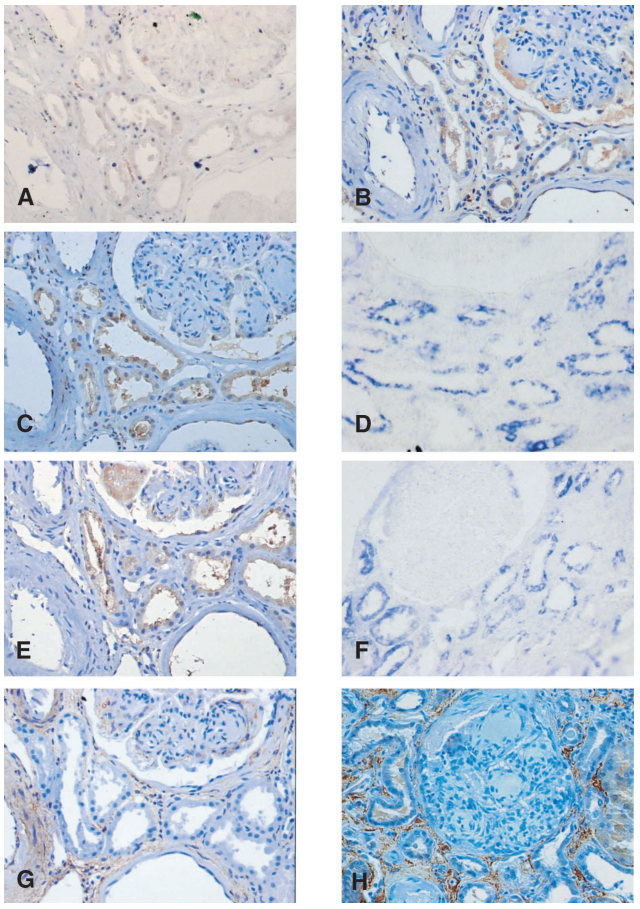


**Fig. 2. Activated nuclear factor kappa B (NFκB) by Southwestern histochemistry, osteopontin (OPN), monocyte chemoattractant protein-1 (MCP-1), and regulated upon activation, normal T cell expressed and secreted (RANTES) (protein and mRNA) in human diabetic nephropathy.** Activated NFκB was mainly detected in (*A*) tubular epithelial cells and, to a lesser extent, in some glomerular and interstitial cells. (*B*) Osteopontin, (*C* and *D*) MCP-1, (*E* and *F*) RANTES protein, and mRNA expressions were detected in the same tubular cells. Immunohistochemical staining for (*G*) α-smooth muscle actin denotes interstitial myofibroblasts, and (*H*) angiotensin-converting enzyme (ACE) neoinduction appears in interstitial cells. The different techniques were performed as described in **Methods**.

In the whole population of patients, the staining score for MCP-1 and RANTES was significantly correlated with that for NFκB tubular activation ($r = 0.7$; $P < 0.01$). As noted in serial sections, the same tubular cells expressed activated NFκB in the nuclei and MCP-1 and RANTES in the cytoplasm (Fig. 2).

### Interstitial inflammatory cells are detected in DN

The composition of the inflammatory cell infiltration was further examined by immunohistochemistry with a specific anti-monocyte/macrophage CD68, anti-lymphocyte CD4, anti-lymphocyte CD8, and anti-α-smooth muscle actin (interstitial myofibroblasts). As the interstitial cell infiltration was found in almost all cases, a great number of cells were recognized as macrophages/monocytes, according to the immunostaining for CD68. However, in cryostat sections, an increased number of CD4+ cells, and, to a lesser extent, CD8+ cells were also found in the interstitium (not shown). Furthermore, positive staining for interstitial myofibroblasts (α-actin–positive cells) was found, particularly in areas of fibrosis (Fig. 2G). In these patients there was a significant association between the interstitial cell infiltration and fibrosis and the staining score for NFκB ($P < 0.05$, Fisher test).

### Association of renal RAS components and inflammatory parameters in DN

In serial sections of different cases, the ACE overexpression and neoinduction were coincidental with the

tubular activation of NFκB, MCP-1, and osteopontin, as well as with the interstitial infiltration and myofibroblast activation as it is shown in Figures 1 and 2.

## DISCUSSION

The tubulointerstitial injury is a major feature of DN and an important predictor of renal dysfunction. Our study reveals that renal biopsies from patients with DN present elevated levels of RAS components (increased ACE and Ang II expression in tubular and interstitial cells and $AT_2$ receptors in tubular cells), NFκB activation, and up-regulation of NFκB-dependent proinflammatory genes (MCP-1, RANTES) were correlated with the magnitude of proteinuria and the interstitial cell infiltration.

Activation of NFκB has been recently found in the kidney of streptozotocin-induced diabetes [20] and in patients with DN [21]. In addition, advanced glycation end products are able to induce oxidative stress and activate NFκB in cultured mesangial cells and tubular cells [21, 22]. Insufficient glycemic control, both in type 1 and type 2 diabetes, increases NFκB binding activity in peripheral blood mononuclear cells [23]. NFκB activation was oxidative stress–sensitive and correlated with the degree of DN [24], thereby strengthening the role of the oxidative stress and NFκB activation in late diabetic complications [25]. Activated NFκB plays an important role in the up-regulation of MCP-1 and RANTES in many cell types [3–8]. In our study, NFκB activity was mainly detected in cortical tubular epithelial cells and, to a lesser extent, in some glomerular cells (mainly podocytes). Some renal interstitial cells, mainly mononuclear cells, also showed NFκB activation. In serial sections we noted that the same tubular epithelial cells simultaneously demonstrated NFκB activation and NFκB-dependent genes (MCP-1 and RANTES), supporting the concept that this transcription factor is involved in the expression of these genes. Studies in diabetic rats have demonstrated an increased glomerular expression of MCP-1 in the early stages of glomerular injury, coinciding with an infiltration of monocyte/macrophages, and both findings were suppressed by the Ang II blockade [26]. In humans, urinary levels of MCP-1 were significantly elevated in patients with diabetic nephrotic syndrome and advanced tubulointerstitial lesions [27], which correlated with the extent of proteinuria [28]. MCP-1 production by cultured human mesangial cells is facilitated by a diabetic milieu [28], and MCP-1–positive cells were detected in the interstitium of advanced DN [27]. Our results, obtained in diabetic patients with advanced glomerular and interstitial lesions, support and extend those observations, confirming that tubular epithelial cells are the main source of MCP-1.

The molecular mechanisms involved in NFκB activation and over-expression of related chemokines are not completely elucidated. At present we cannot discern how much of the NFκB activation is due to the increased trafficking of proteins in the tubular cells [8, 17, 29, 30] or to the diabetic condition, per se, or both. High extracellular glucose activates intracellular signaling cascades, such as NFκB, and alters the expression of cytokines and growth factors, which play a key role in the development of renal damage [31]. On the other hand, the mechanisms by which proteinuria could cause interstitial inflammation and fibrosis are still not fully understood. The incubation of tubular epithelial cells with albumin, in concentrations similar to those found in the urine from patients with nephrotic syndrome, elicited an increment in the NFκB activity [4, 5] that required $H_2O_2$ through a protein kinase C (PKC)-dependent pathway [32]. In models of renal injury with heavy and sustained proteinuria, an over-expression of chemokines [29], as well as of vasoactive peptides (Ang II and endothelin-1) was noted [33].

The data presented here show another potential mechanism that can contribute to the progression of renal damage in DN—the activation of renal RAS. Studies using either ACE inhibitors or $AT_1$ receptor blockers strongly suggest that Ang II is a mediator of progressive injury in DN [11, 34, 35].

Measurement of RAS components in plasma and experimental diabetes have generally shown suppression of the system [11]. In early streptozotozin-induced (STZ) diabetes, a model of type 1 diabetes, glomerular renin, and ACE were elevated [36], while in another study, no changes were observed [37]. Previous studies attempting to measure intrarenal levels of Ang II have disclosed inconsistent results [11]. In the present paper, by means of an anti-Ang II antibody largely used [18], we have noted elevated Ang II immunostaing in the kidneys of DN, mainly in tubular and interstitial cells.

We have also found elevated ACE in tubular and interstitial cells. ACE is up-regulated in proximal tubular epithelium in some kidney diseases [33, 38, 39]. Tubular cells are direct targets for high glucose levels present in diabetes. Indeed, cultured tubular cells exposed to glucose increased angiotensinogen gene expression and Ang II secretion via a glucose-responsive element on the angiotensinogen promoter [40]. ACE over-expression was also observed in renal fibroblast–like cells in the diabetic kidney. In a model of fibrosing kidney, alterations of RAS components have been described [41]. In renal interstitial fibroblasts, all components of RAS are expressed [41, 42] and are activated in response to Ang II, increasing cell proliferation, fibrosis, and expression of angiotensinogen [43]. In patients with DN 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. These data, showing elevated renal Ang II production in the diabetic kidney, could explain the observed beneficial effects of RAS blockers in large clinical trials.

In DN patients, we have found significantly low $AT_1$ receptor levels. A decrease in $AT_1$ mRNA has also been demonstrated in whole kidney biopsy samples from humans with type 2 diabetes [43]. In early STZ diabetes the $AT_1$ gene and protein is also down-regulated [44]. By contrast, in another experimental model of diabetes an $AT_1$, over-expression was described [37]. Although $AT_1$ is mostly down-regulated, there is an in vivo evidence for enhanced sensitivity to Ang II in the diabetic kidney. A potential explanation could be extrapolated from studies in cultured vascular smooth muscle cells (VSMC), which showed that high glucose levels augmented Ang II responses such as cell proliferation, and signaling sytems, including Janus kinase (JAK), signal transducers and activators of transcription (STAT), and NFκB [31, 45]. Interestingly, we have observed that $AT_2$ receptors are up-regulated in human DN. In our study, no signal was found in glomeruli, as previously described the in normal kidney [46]. In an experimental model of early STZ, glomerular $AT_2$ expression was down-regulated [37], showing different responses in human and experimental models of diabetes. $AT_2$ receptor overexpression, mainly in tubulointerstitium, has been described in several models of renal injury, such as Ang II infusion, sodium depletion, and renal ischemia reperfusion [46, 47]. $AT_2$ functions, including vasodilatation, renal natriuresis, and inflammatory cell infiltration [15] are well known, but the role of $AT_2$ in DN remains to be elucidated. The possibility that Ang II sensitivity observed in diabetes could be mediated by $AT_2$ is an important point that needs to be addressed.

Inflammatory cells in the diabetic kidney contain elevated levels of Ang II. Production of Ang II by immunocompetent cells has also been described in animal models of salt-sensitive hypertension [18]. Ang II can activate immune cells, causing chemotaxis, proliferation, differentiation, and production of chemokines [15]. Macrophages and lymphocytes, which are the main cell types in the interstitial cell infiltration, are capable of generating ROS and Ang II [18], and therefore Ang II generated by inflammatory cells can contribute to the perpetuation of the damage.

One potential mechanism that may explain NFκB activation found in human DN is the local Ang II production. In vivo and in vitro Ang II activates NFκB and up-regulates NFκB-related genes [15]. In glomerular mesangial cells, endothelial cells, and VSMC, NFκB activation occurs both via $AT_1$ and $AT_2$ [7, 48–50], while in tubuloepithelial cells it only occurs through $AT_1$ [7]. The $AT_2$/NFκB pathway has also been demonstrated by specific $AT_2$ agonists, and more recently in COS7 cells transfected with $AT_2$ receptors and PC12 cells, that only express $AT_2$ [50]. As the $AT_2$ participates in the inflammatory response via the NFκB pathway, and proinflammatory genes such as RANTES could be triggered via $AT_2$/NFκB [15], it is tempting to speculate that the inflammatory cell infiltration observed in advanced DN could be induced by this pathway. Excess glucose, together with elevated Ang II could contribute to the renal damage. In VSMC, Ang II and glucose synergistically increase NFκB activation [45]. All of these data show that Ang II, through the activation of the NFκB pathway and up-regulation of related genes, mainly in tubular cells, could contribute to renal damage progression in human DN.

## CONCLUSION

Our study clearly shows that components of the intrarenal RAS are selectively activated in the human diabetic kidney. The simultaneous occurrence of interstitial cell infiltration and renin-angiotensin activation suggests that Ang II seems to be a key element in the renal inflammation in diabetic patients.

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