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Vimentin and heat shock protein expression are induced in the kidney by angiotensin and by nitric oxide inhibition

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Background. Angiotensin II (Ang II) infusion and nitric oxide synthesis (NOS) inhibition with N^{ω}-nitro-L-arginine-methyl-ester (L-NAME) are experimental models of hypertension associated with renal inflammation and oxidative stress. To gain insight into the nature of the tubulointerstitial injury induced in these models, we studied lectin-binding specificities, vimentin expression, and heat shock protein (HSP) 60 and 70 in these experimental models.

Methods. Sprague-Dawley rats received Ang II infusion (435 ng/kg/min) for 2 weeks by subcutaneous minipumps (Ang II group, $N = 5$) or L-NAME in the drinking water (70 mg/100 mL) for 3 weeks (L-NAME group $N = 7$). The control group consisted of 10 rats. Systolic blood pressure (tail-cuff plethysmography), serum creatinine, and proteinuria were determined weekly. At the end of the treatment period, rats were sacrificed and kidneys studied. Binding specificities of fluorescein-labeled lectins were examined in frozen sections, and cellular infiltrates were identified by immunohistology and expression of vimentin and HSP 60 and 70 with immunohistochemistry and computer image analysis.

Results. Tubulointerstitial accumulation of macrophages, lymphocytes, and Ang II–positive cells were present in the Ang II group and L-NAME group. Vimentin, HSP 60, and HSP 70 were increased 8 to 20 times in the cortex of the rats of the Ang II group and the L-NAME groups. Neoexpression of vimentin and HSPs was found primarily in proximal tubular cells.

Conclusion. Ang II infusion and NOS inhibition induce tubular injury with epithelial cell transdifferentiation and expression of stress proteins. The role of these changes in the accumulation and activation of the interstitial inflammatory infiltrate merits further investigation.

The administration of angiotensin II (Ang II) by subcutaneous minipumps and the inhibition of nitric oxide (NOS) synthesis by ingestion of N^{ω}-nitro-L-arginine methyl-ester (L-NAME) are experimental protocols that induce well-known models of arterial hypertension in rats [1, 2, 3]. This hypertension is reversible. Shortly after these treatments are stopped the blood pressure returns to normal and remains within normal limits if the dietary content of sodium is restricted. However, the administration of a high-salt diet following these experimental manipulations results in a progressive rise of blood pressure.

The pathogenesis of the post-Ang II and post-L-NAME salt-sensitive hypertension has been studied by our group. A key feature appears to be the infiltration of lymphocytes and macrophages into the renal interstitium that generates both Ang II and oxidants [4, 5]. Preventing the infiltration by administering immunosuppressive therapy with mycophenolate mofetil (MMF) will prevent or attenuate the salt-driven hypertension [4, 5]. The importance of the interstitial infiltrate in the genesis of a hypertension induced by a high-salt diet has also been corroborated in other experimental models, such as protein overload proteinuria [6], and in genetically hypertensive rats [7–9]. The mechanism appears to involve persistent renal vasoconstriction [10], which may be mediated by the Ang II expressed by the inflammatory cells as a consequence of oxidant-mediated inactivation of nitric oxide (NO) and endothelial dysfunction [11–13].

Modifications of normal structural components, phenotypic changes, exposure of neoantigens, and tubular injury and regeneration are likely to occur during the period of Ang II infusion and NOS synthesis inhibition. These events are potentially important because they could be at the root of the reactivity that results in the infiltration of lymphocytes and monocytes in tubulointerstitial areas of the kidney. Therefore, we investigated if changes in lectin-binding specificities, vimentin expression, and induction of heat shock protein (HSP) 60, predominantly expressed in the cortex, and HSP 70, predominantly expressed in the medulla [14], occur as a result of the Ang II infusion and NOS inhibition protocols that result in subsequent salt-sensitive hypertension.

METHODS

Experiments were done in male Sprague-Dawley rats weighing 290 to 340 g obtained from the Instituto Nacio-

nal de Investigaciones Científicas (Los Teques, Venezuela). They had free access to water and received standard rat chow with normal (0.4% NaCl) sodium content (Protinal, Valencia, Venezuela). Details of the experimental protocols of Ang II infusion and NOS inhibition models have been previously reported [4, 5]. Briefly, the Ang II infusion model (Ang II group, $N = 5$) consisted in the administration of 435 ng/kg/min of Ang II (Sigma Chemical Co., St. Louis, MO, USA) during 2 weeks by surgically placed subcutaneous minipumps (Alza Corporation, Palo Alto, CA, USA). NOS inhibition (L-NAME group, $N = 7$) was achieved by the administration of L-NAME in the drinking water (70 mg/100 mL) for 3 weeks. The control group consisted of 10 rats.

At the end of the experimental period rats were euthanized under penthotal anesthesia and the kidneys were harvested for histology and immunohistology.

Systolic blood pressure (SBP) was determined weekly by tail-cuff plethysmography (IITC; Life Scientific Instruments, Woodland Hills, CA, USA) in rats pre-conditioned to the procedure. Serum creatinine and proteinuria were determined weekly.

Histology and immunohistology

Light microscopy evaluation was done in 4 μm sections of paraffin-embedded tissues stained with periodic acid-Schiff reagent (PAS) and Trichrome stains using a semiquantitative glomerular sclerosis index [15] and tubulointerstitial damage score, as previously reported [4, 5].

Macrophage and lymphocyte infiltration was evaluated by indirect immunofluorescence using monoclonal antibodies (see later). Cellular counts in glomeruli were expressed as positive cells with a given staining/glomerular cross-section (gcs), and in the tubulointerstitium as positive cells/mm^2.

Vimentin and HSP 60 and 70 were investigated by the immunoperoxidase technique, as described previously [16] using the appropriate mAbs (see later). Vimentin expression was used as a marker of tubulointerstitial injury and epithelial/mesenchymal transdiffferentiation. Expression of vimentin and HSPs was studied with computer-assisted analysis of digitalized images acquired with a Zeiss Axioscope (Gottingen, Germany) fitted with a Kodak DC 120 digital megapixel camera as described before [1, 5]. Vimentin and HSP image analysis was done in at least 5 separate sections studied under low power of each kidney. Results of image analysis are expressed as the ratio of positive areas (areas expressing a given element) over the total area examined. Obviously damaged areas were excluded from computer-assisted image analysis. All histologic studies were done in a blinded fashion.

Antisera

Anti ED-1 (monoclonal antibody to macrophages; Harlan Bioproducts, Indianapolis, IN, USA) and anti-CD5 (clone MRCOX19; Biosource International, Camarillo, CA, USA) were used to identify macrophages and lymphocytes. Vimentin was identified with mouse antirat vimentin (clone V9; Dako Corp., Carpinteria, CA, USA). HSP 60 was identified with polyclonal rabbit anti-HSP 60 antibody (Accurate Chemical & Scientific Corp., Westbury, NY, USA). HSP 70 was identified with polyclonal rabbit anti-HSP70 (Hsp72) (Stressgen Biotechnologies, Victoria, Canada)

Secondary rat antimouse and donkey antirabbit antibodies were purchased from Accurate Chemical and Scientific Corporation.

Lectins

Lectin binding was studied in frozen sections and it was graded with an arbitrary intensity scale (0 to 4) in the various renal structures. The following fluorescein-labeled lectins were tested: *Bandeiraea simplicifolia* (carbohydrate specificity, -D-Galactose-D-Glucose N-acetyl); *Glycine Max* (carbohydrate specificity, -D-Galactose N-acetyl-D-Galactose); *Dolichus biflorus* (carbohydrate specificity, -D-Galactose N-acetyl); and *Arachys hypogaea* (carbohydrate specificity, -D-Galactose-(1-3) D-Galactose N-acetyl). Normal binding of the various lectins to the kidney structures was examined in control biopsies and it was found to agree with the report of Holtofer et al [17].

Statistical analysis

Comparison between groups were done by one-step analysis of variance (ANOVA) with Tukey-Kramer post-tests. Two-tailed P values are used throughout and $P < 0.05$ were considered statistically significant. Results throughout the paper are given as mean ± SD.

RESULTS

At the end of the experiment the rats receiving Ang II or L-NAME were hypertensive. The Ang II group had SBP of 228 ± SD, 20 mm Hg, and the L-NAME group had SBP of 194 ± 24 mm Hg. Control rats remained normotensive (SBP = 128 ± 18).

Baseline serum creatinine (mg/dL) was 0.33 ± 0.08 in the Ang II group and 0.30 ± 0.1 in the L-NAME group. At the end of the experiment it had increased to 0.72 ± 0.12 ($P < 0.05$) in the Ang II group and was unchanged in the L-NAME group (0.4 ± 0.1). Proteinuria remained within normal limits during the experiment (<10 mg/24 h).

Histology and cellular infiltration

The glomerular histologic damage was minor in the Ang II group and consisted of occasional glomeruli showing focal glomerulosclerosis and mesangial expansion. In the L-NAME group, the glomerulosclerosis index (0 to 400) [15] was 75 ± 18. Tubulointerstitial damage score

Table 1. Lectin-binding characteristics

	GLOM	BC	PT	DT	CD	TBM	BB	V
DOLICHUS BIFLORUS								
Sham	0	0	1.1 ± 0.7	1.1 ± 0.7	0	0	0	0
Ang II	0.2	0	1.2 ± 0.7	2.0 ± 0.7	0	0	0	0
L-NAME	0	0	1.1 ± 0.7	2.0 ± 0.7	0	0	0	0
ARACHYS HYPOGEA								
Sham	0.3 ± 0.3	1.4 ± 1.4	0.2 ± 0.2	2.0 ± 0.0	1.8 ± 0.4	0.4 ± 0.5	0.8 ± 0.8	0.8 ± 0.7
Ang II	0.0 ± 0.3	1.4 ± 0.9	0.7 ± 0.3	1.6 ± 0.5	2.0 ± 0.0	0.7 ± 0.4	0.4 ± 0.4	1.0 ± 0.0
L-NAME	0	1.6 ± 0.6	0.5 ± 0.3	1.8 ± 0.4	1.8 ± 0.4	0.6 ± 0.4	0.7 ± 0.8	1.0 ± 0.2
GLYCINE MAX								
Sham	0	0	0.8 ± 0.4	1.6 ± 0.5	2.8 ± 0.8	0	0.4 ± 0.6	0
Ang II	0	0	0	2.0 ± 0.0	2.4 ± 0.2	0	1.0 ± 0.7	0.5 ± 0.7
L-NAME	0	0	0	2.0 ± 0.5	2.4 ± 0.2	0	0.9 ± 0.6	0
ANDEIRAEA SIMPLICIFOLIA								
Sham	2.3 ± 0.9	0	2.0 ± 0.4	1.8 ± 0.4	1.4 ± 0.5	0	1.5 ± 1.5	0
Ang II	1.4 ± 0.5	0	0	1.6 ± 0.5	2.0 ± 0.0	0	2.5 ± 0.6	0
L-NAME	1.8 ± 0.6	0	1.5 ± 1.2	1.5 ± 0.8	2.1 ± 1.2	0	2.0 ± 0.9	0

Abbreviations are: BB, brush border; BC, Bowman's capsule; CD, collecting duct; DT, distal tubule; GLOM, glomerular tuft; PT, proximal tubule; TBM, tubular basement membrane; V, vessels (arterioles). Positive lectin binding graded on a scale of 0 to 4.

(0 to 5) was 2.9 ± 0.7 in the Ang II group and 1.3 ± 0.7 in the L-NAME group (P = NS).

Infiltration of macrophages, lymphocytes, and Ang II positive cells in tubulointerstitial areas was present in the experimental groups. Macrophages (ED1-positive cells/mm^2) were increased ($P < 0.05$) in the rats from Ang II group (44 ± 34) and in the rats from the L-NAME group (38 ± 17) in relation to the values in the control group (12 ± 4). Lymphocyte infiltration was as follows: control group, 8.8 ± 4.4 CD5-positive cells/mm^2; Ang II group, 65 ± 37 ($P < 0.05$ vs. control); and L-NAME, 106 ± 62 ($P < 0.001$ vs. controls).

Ang II–positive cells were seldom seen in the rats from the control group. In the experimental groups they were present in the tubulointerstitial areas: Ang II group, 44 ± 5 positive cells/mm^2 and L-NAME group, 38 ± 17.

Lectin binding

Lectin-binding characteristics were not significantly altered by Ang II infusion or by NOS synthesis inhibition. Results are shown in Table 1.

Vimentin and HSP expression

Relevant changes in the neoexpression of vimentin, HSP 60, and HSP 70 were restricted to the tubulointerstitial areas. Figure 1 shows how vimentin and HSP 60 and HSP 70 were significantly over-expressed in the Ang II group and in the L-NAME group.

Normally, vimentin is expressed only in glomeruli, arterioles, and interstitial fibroblasts (Fig. 2A), and the proximal tubules are negative. Vimentin neoexpression was demonstrated in the renal cortex of the Ang II group and the L-NAME group, particularly in proximal tubules and arterioles (Fig. 2B)

Expression of HSP 70 was increased in both the Ang II group and in the L-NAME group in the proximal tubules and collecting ducts in cortex (Fig. 2 C and D). The variability of the image analysis results in the medullary region was large and there were no significant differences between the experimental and control groups. HSP 60 was over-expressed in the proximal tubules of the renal cortex in both experimental groups.

DISCUSSION

Ang II infusion and L-NAME administration were associated with severe arterial hypertension as previously reported by other groups [1, 2, 3] beside our own [4, 5]. The macrophage and lymphocyte interstitial infiltration associated with these experimental models has also been reported in previous studies [1, 2–5].

There were no changes in the lectin-binding patterns after Ang II infusion and L-NAME administration. These findings are similar to those reported by Eddy [18] in overload proteinuria, in which intense tubulointerstitial accumulation of immune cells occur but the lectin-binding specificity was unmodified.

Vimentin is a widely expressed intermediate filament protein of the cytoskeleton. Vimentin filament segregation is controlled, at least in part, by the protein kinase Aurora-B, which regulates vimentin phosphorylation [19]. Over-expression of vimentin is a marker of tubulointerstitial injury and regeneration with epithelial-mesenchymal transdifferentiation [20]. Neoexpression of vimentin has been used to define renal injury in a variety of renal conditions, such as the non-clipped kidney in the two-kidney one-clip Goldblatt hypertension [21], ureteral obstruction [22], severe proteinuria [18, 23], aging [24], and adriamycin nephropathy [25, 26]. Recent investigations indicate that activated macrophages secrete vimentin [27]. These findings are of interest since the macrophage infiltration is a constant feature after angiotensin

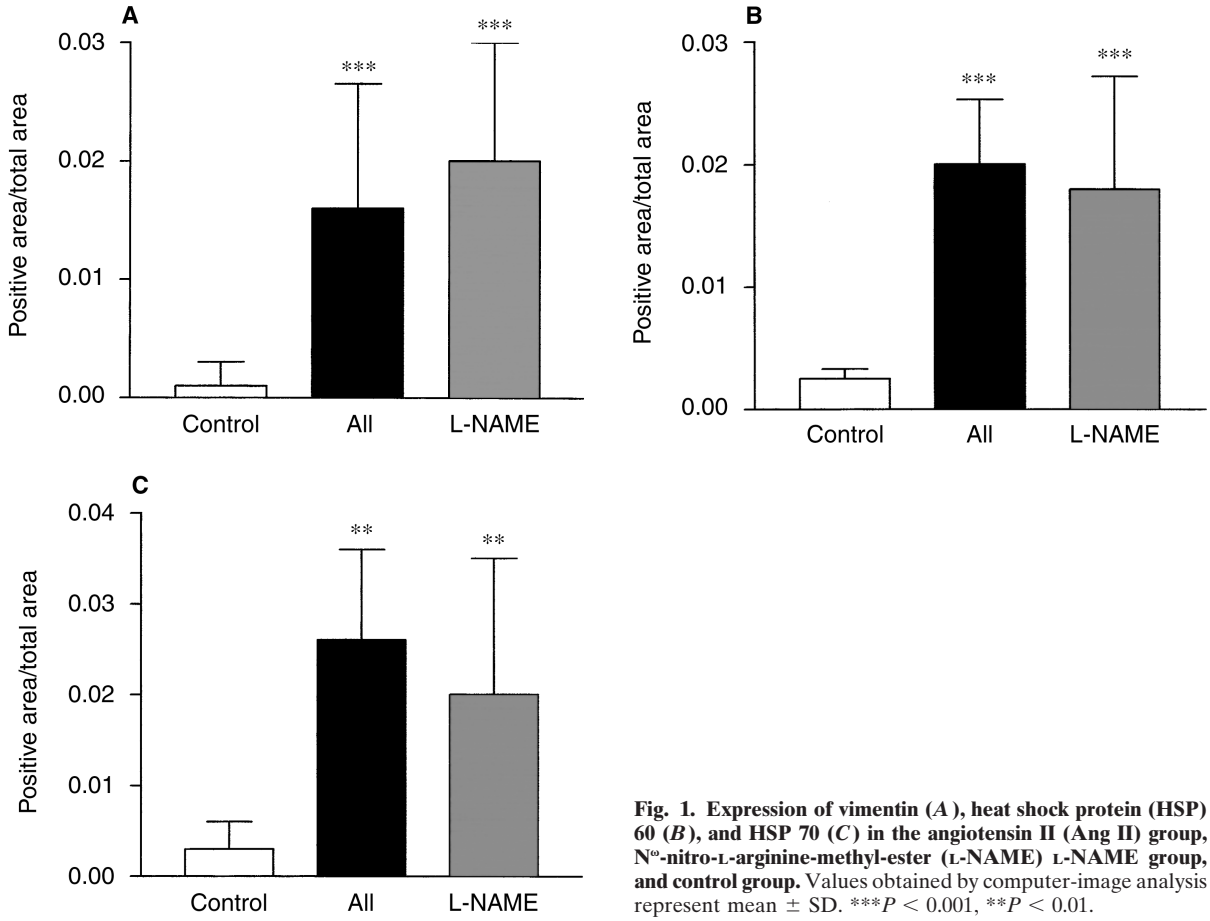

Fig. 1. Expression of vimentin (*A*), heat shock protein (HSP) 60 (*B*), and HSP 70 (*C*) in the angiotensin II (Ang II) group, N^{ω}-nitro-L-arginine-methyl-ester (L-NAME) L-NAME group, and control group. Values obtained by computer-image analysis represent mean ± SD. ***$P < 0.001$, **$P < 0.01$.

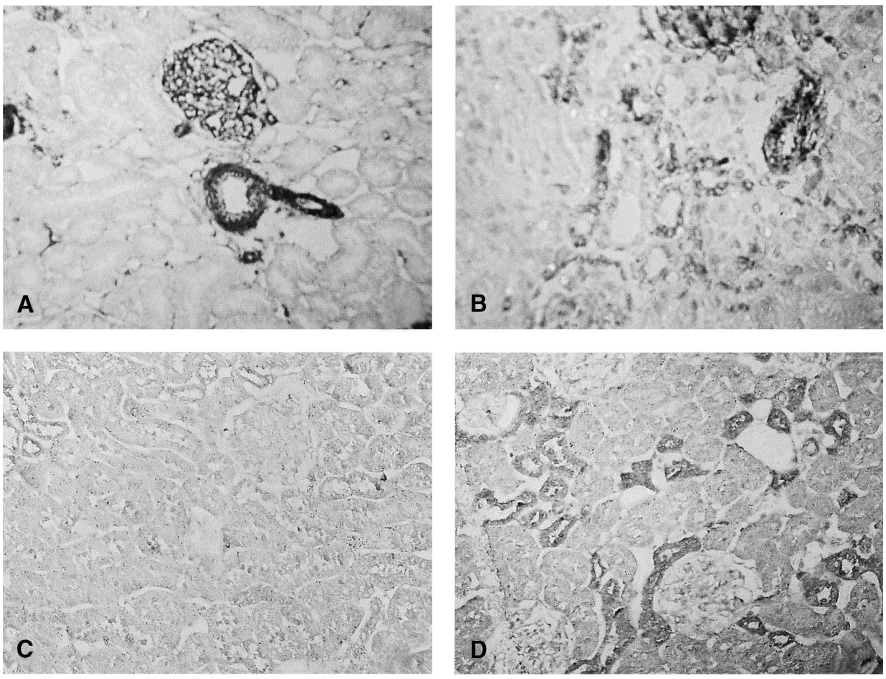

Fig. 2. Immunohistochemistry studies show the normal expression of vimentin (*A*) in the glomeruli, arterial wall, and in occasional interstitial fibroblasts. Intense neoexpression of vimentin is observed in the proximal tubules in a rat from the angiotensin II (Ang II) group (*B*). Over-expression of heat shock protein (HSP) 70 in rats from the Ang II group (*D*) is evident in comparison with the control group (*C*).

infusion [1] and nitric oxide synthesis inhibition [3]. Transdifferentiation of tubular epithelial cells into fibroblasts/myofibroblasts is a process regulated by transforming growth factor β (TGFβ) through the Smad signaling pathway, which is also known to be stimulated by Ang II [20]. Work by Kobayashi et al [21] indicates that angiotensin II, interacting with interferon growth factor (IGF), may influence tubulointerstitial cell kinetics and induce vimentin expression. This work is particularly relevant to our findings from investigations that have shown that Ang II is produced by both infiltrating and tubulointerstitial cells as a consequence of both Ang II infusion and L-NAME administration [4, 5]; therefore, Ang II may participate in the epithelial mesenchymal transdifferentiation characterized by vimentin neoexpression in these experimental models. At any rate, the neoexpression of vimentin in the proximal tubules is an unequivocal signal of injury and regeneration in these areas.

Heat shock proteins are produced as an immediate cellular response to environmental stresses such as heat, oxidants, or ATP depletion [14]. HSP 60 and 70, among others, are present in the kidney [28, 29]. HSP 60 is predominantly expressed in normal kidneys in the cortex and outer medulla [14], its intrarenal distribution roughly corresponding with the abundance of mitochondria [30]. Immunohistochemical studies [29–31] have shown significant positive reactions in proximal tubular cells and to a lesser extent in distal tubular cells. In contrast, HSP 70/72 tissue content increases toward the medulla [29, 31] in a pattern similar to that of the intrarenal solute-concentration gradient. Intense immunostaining for HSP 70 is normally found in epithelial papillary cells and collecting ducts [29].

Ang II is known to induce over-expression of HSP 70, HSP 25, HSP 32, and heme-oxygenase 1 (HO-1) in the rat kidney [32, 33]. Using serial sections, Ishizaka et al [33] observed over-expression of HSP 70 in the same proximal tubules in the cortex that over-expressed HSP 25 and HO-1. They concluded that Ang II triggered similar mechanisms for up-regulation of HSP 70 and HSP 25, and that their increase was dependent on Ang II–receptor type I activation. In the present studies we also found significant increments in the expression of HSP 70 as a result of Ang II infusion and L-NAME administration in proximal tubules and collecting ducts in the renal cortex (Figs. 1 and 2 C and D). In addition, we also found that the expression of HSP 60 was increased by NOS synthesis inhibition. Positive areas were particularly evident in the proximal tubular epithelium.

HSP plays a protective role against cell damage; therefore, our findings suggest that stressful signals resulting from Ang II and NOS inhibition are originating in the renal cortex. In this regard, it is interesting that HSP protects cells from the toxic effect of reactive oxygen species (ROS) [34], and oxidative stress, as determined by the existence of superoxide-positive cells, has been observed in cortical areas of the kidney in these experimental models [4, 5].

The increased expression of HSP raises the question of the participation of these HSPs in the development and expansion of the inflammatory infiltrate. HSP may directly promote leukocyte accumulation since several studies have shown that HSP induces pro-inflammatory cytokine production, as well as over-expression of adhesion molecules E-selectin, ICAM-1, and VCAM-1 [35–38]. In addition, HSPs may promote antigen-specific immune reactivity since they bind peptides in damaged tissue to form HSP-peptide complexes with a strong immunogenic potential [38–40]. In fact, recent work has emphasized that HSPs have a role in antigen presentation [41] and may act as activators of the innate immune system [42, 43]

CONCLUSION

We have shown that phenotypic changes characterized by vimentin neoexpression and stress-related production of HSP 60 and HSP 70 take place in the kidney as a result of Ang II infusion and L-NAME. The location of these changes corresponds to the tubulointerstitial areas where the existence of inflammation and oxidative stress have been linked to the development of salt sensitive hypertension [4, 5]. Further studies are needed to clarify the relationship between these factors.

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REFERENCES

1. Lombardi D, Gordon KL, Polinsky P, *et al*: Salt-sensitive hypertension develops after short-term exposure to angiotensin II. *Hypertension* 33:1013–1019, 1999
2. Baylis C, Mitruka B, Deng A: Chronic blockade of nitric oxide synthesis in the rat produces systemic hypertension and glomerular damage. *J Clin Invest* 90:278–281, 1992
3. Oliveira M, Antunes E, de Nucci G, *et al*: Chronic inhibition of nitric oxide synthesis. A new model of arterial hypertension. *Hypertension* 20:298–303, 1992
4. Rodríguez-Iturbe B, Pons H, Quiroz Y, *et al*: Mycophenolate mofetil prevents salt-sensitive hypertension resulting from angiotensin II exposure. *Kidney Int* 59:2222–2232, 2001
5. Quiroz Y, Pons H, Gordon KI, *et al*: Mycophenolate mofetil prevents the salt-sensitive hipertension resulting from short-term nitric oxide synthesis inhibition. *Am J Physiol Renal Physiol* 281: F38–F47, 2001
6. Alvarez V, Quiroz Y, Nava M, Rodriguez-Iturbe B: Overload proteinuria is followed by salt-sensitive hipertension caused by renal infiltration of immune cells. *Am J Physiol Renal Physiol* 283: F1132–F1141, 2002
7. Rodriguez-Iturbe B, Quiroz Y, Nava M, *et al*: Reduction of renal immune cell infiltration results in blood pressure control in

genetically hypertensive rats. *Am J Physiol Renal Physiol* 282: F191–F201, 2002
8. Nava M, Quiroz Y, Vaziri ND, Rodríguez-Iturbe B: Melatonin reduces renal interstitial inflammation and improves hypertension in spontaneously hypertensive rats. *Am J Physiol Renal Physiol* 284:F447–F454, 2003
9. Rodríguez-Iturbe B, Zhan C-De, Quiroz Y, *et al*: Antioxidant-rich diet improves hypertension and reduces renal immune infiltration in spontaneously hypertensive rats. *Hypertension* 41:341–346, 2003
10. Franco M, Tapia E, Santamaría J, *et al*: Renal cortical vasoconstriction contributes to the development of salt-sensitive hypertension after Angiotensin II exposure. *J Am Soc Nephrol* 12:2263–2271, 2001
11. Rodríguez-Iturbe B, Quiroz Y, Herrera-Acosta J, *et al*: The role of immune cells infiltrating the kidney in the pathogenesis of salt-sensitive hypertension. *J Hypertension* 20(Suppl 3):S9–S14, 2002
12. Johnson RJ, Rodríguez-Iturbe B, Schreiner G, Herrera-Acosta J: Hypertension: A microvascular and tubulointerstitial disease. *J Hypertens* 20(Suppl 3):S1–S7, 2002
13. Johnson RJ, Herrera J, Schreiner G, Rodríguez-Iturbe B: Acquired and subtle renal injury as a mechanism for salt-sensitive hypertension: Bridging the hypothesis of Goldblatt and Guyton. *N Engl J Med* 346:913–923, 2002
14. Beck F-X, Neuhofer W, Müller E: Molecular chaperones in the kidney: distribution, putative roles and regulation. *Am J Physiol Renal Physiol* 279:F203–F215, 2000
15. Raij L, Azar S, Keane W: Mesangial immune injury, hypertension and progressive damage in Dahl rats. *Kidney Int* 26:137–143, 1984
16. Parra G, Moreno P, Rodríguez-Iturbe B: Glomerular proliferative activity and t lymphocyte infiltration in acute serum sickness. *Clin Immunol Immunopathol* 82:299–302, 1997
17. Holthofer J, Virtanen I, Petterson E, *et al*: Lectins as fluorescence microscopic markers for saccharides in the human kidney. *Lab Invest* 45:391–399, 1981
18. Eddy AA: Interstitial nephritis induced by protein overload proteinuria. *Am J Pathol* 35:719–731, 1989
19. Goto H, Yasui Y, Kawajiri A, *et al*: Aurora-B regulates the cleavage furrow-specific vimentin phosphorilation in the cytokinetic process. *J Biol Chem* 278:8526–2530, 2003
20. Lan HY: Tubular epithelial-myofibroblast transdifferentiation mechanisms in proximal tubules. *Curr Opin Nephrol Hypertens* 12:25–29, 2003
21. Kobayashi S, Ishida A, Moriya H, *et al*: Angiotensin II receptor blockade limits kidney injury in two-kidney one-clip Goldblatt hypertensive rats with special reference to phenotypic changes. *J Lab Clin Med* 133:134–143, 1999
22. Chevalier RL, Goyal S, Kim A, *et al*: Renal tubulointerstitial injury from ureteral obstruction in the neonatal rat is attenuated by IGF-1. *Kidney Int* 57:882–890, 2000
23. Kikuchi H, Kawachi H, Ito Y, *et al*: Severe proteinuria, sustained for 6 months, induces tubular epithelial cell injury and cell infiltration in rats but not progressive interstitial fibrosis. *Nephrol Dial Transplant* 15:799–810, 2000
24. Nakatsuji S, Yamate J, Sakuma S: Relationship between vimentin expressing renal tubules and interstitial fibrosis in chronic progressive nephropathy in aged rats. *Virchows Arch* 433:359–367, 1998
25. Galli LG, Volpini RA, Costa RS, *et al*: Tubular cell lesion, albuminuria and renal albumin handling in rats treated with adriamycin. *Ren Fail* 23:693–703, 2001
26. Shu Y, Hoshi S, Tomari S, *et al*: Phenotypic changes and cell activation in early tubulointerstitial injury of rat adriamycin nephrosis. *Pathol Int* 52:214–223, 2002
27. Mor-Vaknin N, Punturieri A, Sitwala K, Markowitz DM: Vimentin is secreted by activated macrophages. *Nat Cell Biol* 5: 59–63, 2003
28. Pelham H: Heat-shock proteins: Coming in from the cold. *Nature* 332:776–777, 1988
29. Müller E, Neuhofer W, Ohno A, *et al*: Heat shock proteins HSP25, HSP60, HSP72, HSP73 in isoosmotic cortex and hyperosmotic medulla of rat kidney. *Pflugers Arch* 431:608–617, 1996
30. Pfaller W: Structure function correlations in the rat kidney. *Adv Anat Embryol Cell Biol* 70:1–106, 1982
31. Hernández-Pando R, Pedraza-Chaverri J, Orozco-Estevez H, *et al*: Histological and subcellular distribution of 65 and 70 kD heat shock proteins in experimental nephrotoxic injury. *Exp Toxic Pathol* 47:501–508, 1995
32. Aizawa T, Ishizaka N, Taguchi J, *et al*: Heme-oxygenase 1 is upregulated in the kidney of angiotensin II-induced hypertensive rats: Possible role in renoprotection. *Hypertension* 35:800–806, 2000
33. Ishizaka N, Aizawa T, Ohno M, *et al*: Regulation and localization of HSP70 and HSP25 in the kidney of rats undergoing long-term administration of angiotensin II. *Hypertension* 39:122–128, 2002
34. Polla BS, Bachelet M, Elia G, Santoro MG: Stress proteins in inflammation. *Ann N Y Acad Sci* 851:75–85, 1998
35. Galdiero M, de Léro GC, Marcatili A: Cytokine and adhesion molecule expression in humam monocytes and endotelial cells stimulated with bacterial heat shock proteins. *Infect Immun* 65:699–707, 1997
36. Retzlaff C, Yamamoto Y, Hoffman PF, *et al*: Bacterial heat shock proteins directly induce cytokine mRNA and interleukin-1 secretion in macrophage cultures. *Infect Immun* 62:5689–5693, 1994
37. Peetermans WE, Raats CJ, Langermans JA, van Furth R: Mycobacterial heat shock protein 65 induces proinflammatory cytokines but does not activate human mononuclear phagocytes. *Scan J Immunol* 39:613–617, 1994
38. Kol A, Bourcier T, Lichtman A, Libby P: Chlamydial and human heat shock protein 60 activate human vascular endothelium, smooth muscle cells and macrophages. *J Clin Invest* 103:571–577, 1999
39. Cho BK, Palliser D, Guillen E, *et al*: A proposed mechanism for the induction of cytotoxic T lymphocyte production by heat shock fusion proteins. *Immunity* 12:263–272, 2000
40. Udono H, Yamano T, Kawabata E, *et al*: Generation of cytotoxic T lymphocytes by MHC class I ligands fused to heat shock cognate protein 70. *Int Immun* 13:1233–1242, 2001
41. Zihai L, Menoret A, Srivastava P: Roles of heat shock proteins in antigen presentation and cross-presentation. *Curr Opin Immunol* 14:45–51, 2002
42. Multhoff G, Botzler C: Heat-shock response and the immune response. *Ann N Y Acad Sci* 851:86–93, 1998
43. Wallin RPA, Lundqvist A, Moré SH, *et al*: Heat shock proteins as activators of the innate immune system. *Trends Immunol* 23: 130–135, 2002