Renal vascular and tubulointerstitial inflammation and proliferation in Cyp1a1-Ren2 transgenic rats with inducible ANG II-dependent malignant hypertension

Miguel L. Graciano,1 Cynthia R. Mouton,1 Matthew E. Patterson,1 Dale M. Seth,1 John J. Mullins,2 and Kenneth D. Mitchell1

1Department of Physiology, Hypertension and Renal Center of Excellence, Tulane University Health Sciences Center, New Orleans, Louisiana; and 2Centre for Cardiovascular Science, University of Edinburgh Medical School, Edinburgh, United Kingdom

Submitted 27 November 2006; accepted in final form 27 February 2007

Graciano ML, Mouton CR, Patterson ME, Seth DM, Mullins JJ, Mitchell KD. Renal vascular and tubulointerstitial inflammation and proliferation in Cyp1a1-Ren2 transgenic rats with inducible ANG II-dependent malignant hypertension. Am J Physiol Renal Physiol 292: F11006–F11006, 2007. First published March 6, 2007; doi:10.1152/ajprenal.00469.2006.—Transgenic rats with inducible ANG II-dependent malignant hypertension [TGR(Cyp1a1Ren2)] were generated by inserting the mouse Ren2 renin gene into the genome of the rat. The present study was performed to assess renal morphological changes occurring during the development of ANG II-dependent malignant hypertension in these rats. Male Cyp1a1-Ren2 rats (n = 10) were fed normal rat food containing indole-3-carbinol (I3C, 0.3%) for 10 days to induce malignant hypertension. Rats induced with I3C had higher mean arterial pressures (173 ± 9 vs. 112 ± 11 mmHg, P < 0.01) than noninduced normotensive rats (n = 9). Glomerular damage was evaluated by determination of the glomerulosclerosis index (GSI) in tissue sections stained with periodic acid-Schiff. Kidneys of hypertensive rats had a higher GSI than noninduced normotensive rats (21.3 ± 5.6 vs. 3.5 ± 1.31 units). Quantitative analysis of macrophage ED-1-positive cells and proliferating cell nuclear antigen using immunohistochemistry demonstrated increased macrophage numbers in the renal interstitium (106.4 ± 11.4 vs. 58.7 ± 5.0 cells/mm²) and increased proliferating cell number in cortical tubules (37.8 ± 5.7 vs. 24.2 ± 2.1 cells/mm²), renal cortical vessels (2.2 ± 0.5 vs. 0.13 ± 0.07 cells/vessel), and the cortical interstitium (33.6 ± 5.7 vs. 4.2 ± 1.4 cells/mm²) of hypertensive rat kidneys. These findings demonstrate that the renal pathological changes that occur during the development of malignant hypertension in Cyp1a1-Ren2 rats are characterized by inflammation and cellular proliferation in cortical vessels and tubulointerstitium.

Address for reprint requests and other correspondence: K. D. Mitchell, Dept. of Physiology, Tulane University Health Sciences Center, 1430 Tulane Ave., SL39, New Orleans, LA 70112 (e-mail: kdmitch@tulane.edu).

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
In this regard, it has been shown that Cyp1a1-Ren2 rats induced with 0.3% I3C for 14 days exhibit malignant vascular injury with fibrinoid necrosis and endarteritis obliterator of interlobular arteries and afferent arterioles (23). However, the renal inflammatory responses to induction of malignant hypertension were not determined in this initial study. Given that this form of severe hypertension is associated with very rapid and pronounced increases in blood pressure, the renal inflammatory changes in ANG II-dependent malignant hypertension remain uncertain. Nevertheless, in light of the evidence that ANG II-induced activation of inflammatory mediators and immune cells contributes importantly to the pathogenesis of renal tissue injury in various hypertensive states (4, 8, 11, 12, 17, 35, 36, 39), we hypothesized that renal vascular and interstitial inflammation also contribute importantly to the renal injury that occurs in Cyp1a1-Ren2 rats with ANG II-dependent malignant hypertension.

The present study was performed to evaluate the renal morphological changes occurring during the development of ANG II-dependent malignant hypertension in Cyp1a1-Ren2 rats, particularly those related to cortical inflammation and preglomerular arteriolar structure as they might relate to the pathophysiology of this severe form of ANG II-dependent hypertension. Particular emphasis was placed on assessment of the renal histological changes in the various microenvironments of the renal cortex, namely, the glomeruli, tubules, tubular interstitium, and vessel walls, to determine whether they might be differentially affected by the high-renin, high-ANG II, and increased blood pressure environment known to occur in this novel transgenic model (23, 27).

MATERIALS AND METHODS

Experimental groups and blood pressure measurement. The experimental procedures used in this study conformed to the National Institutes of Health Guide for the Care and Use of Laboratory Animals and were approved by the Institutional Animal Care and Use Committee of Tulane University Health Sciences Center. Experiments were performed in adult male Cyp1a1-Ren2 transgenic rats bred at Tulane University Health Sciences Center from stock animals supplied from the University of Edinburgh (Edinburgh, UK). In one group, male Cyp1a1-Ren2 transgenic rats (n = 10) were fed a normal diet containing the aryl hydrocarbon I3C [0.3% wt/wt, diet TD 00554, Harlan-Teklad, Madison, WI] for 10 days to induce malignant hypertension. In a second group (n = 9), age-matched male Cyp1a1-Ren2 rats fed normal rat food (diet TD 90229, Harlan-Teklad), which did not contain I3C, served as controls.

The rats were anesthetized with pentobarbital sodium (50 mg/kg ip), and the abdominal cavity was opened via a midline incision. A polyethylene catheter was inserted into the aorta to allow measurement of arterial blood pressure. The kidneys were excised, fixed in buffered formalin (10% vol/vol), and embedded in paraffin for histological examination as well as immunohistochemical identification of macrophages and proliferating cell activity.

Histology and morphometry. Sections 3-μm thick were stained with periodic acid-Schiff (PAS) reaction. The extent of glomerulosclerosis was evaluated using a simplification of the procedure described by Fujihara et al. (11, 12) by attributing a score to each glomerulus according to the extent of sclerotic injury: 0 = intact glomeruli; 1 = lesions affecting 50% or less of the glomerular area; and 2 = lesions affecting 51–100% of the glomerular area. The glomerulosclerosis index (GSI) was calculated for each rat as the weighted average of all individual glomerular scores thus obtained, multiplied by 100. At least 70 glomeruli were examined for each rat.

The images were digitally recorded using image-acquisition software (Magnafire) coupled with a Nikon BF50 microscope. To validate the examiner-based evaluation of the degree of mesangial expansion, mesangial and total glomerular areas were measured through computer-based analysis. In this case, the glomerulus was defined as the minimal convex polygon surrounding the capillary tufts, and the mesangial compartment was defined by selecting PAS-positive material. The amount of glomerular area filled by PAS-positive material was calculated by computer. The calculation of the mesangial and glomerular areas was performed using image-analysis software (Imagepro plus). The results are expressed as the percentage of the total glomerular area.

Immunohistochemistry. Macrophages and proliferating cells were analyzed in 3-μm-thick paraffin-embedded sections obtained after 10 days of treatment. Sections were mounted on glass slides, deparaffinized in xylene, and rehydrated through graded ethanol and in deionized water in the final step. Sections were then subjected to microwave irradiation in citrate buffer to enhance antigen retrieval and preincubated with 5% normal horse serum in Tris-buffered saline (TBS) to prevent nonspecific protein binding. Negative control experiments for all antigens were performed by omitting incubation with the primary antibody.

To detect proliferating cells, renal sections were analyzed using a monoclonal mouse antibody (Dako) specific for the proliferating cell nuclear antigen (PCNA) and an indirect streptavidin-biotin alkaline phosphatase technique. Sections were preincubated with avidin and biotin solutions to block nonspecific binding of these compounds and then with normal horse serum (Vector Labs, Burlingame, CA) as mentioned. The incubation with the primary antibody was carried overnight at 4°C in a humidified chamber. After being washed, sections were then incubated with rat-adsorbed biotinylated antiamouse IgG (Vector Labs) for 45 min at room temperature, followed by incubation with the streptavidin-biotin-alkaline phosphatase complex (Dako) for 30 min at room temperature. Sections were incubated with a freshly prepared substrate, consisting of naphtholphosphatase, which stains the muscular layer of vessels) was performed. The quantitation of cortical interstitial and tubular PCNA-positive cells was carried out in a blind fashion under 400 magnification and expressed as cells per square millimeter. For each section, 30 microscopic fields, each corresponding to an area of 0.13 mm², were examined. The number of cells located at the glomeruli, tubules, and vessels was also determined and expressed as cells per glomerulus and cells per vessel. The quantitation of cortical interstitial and tubular ED-1-positive cells in the interstitium was expressed as cells per square millimeter. Again, 30 microscopic fields, each corresponding to an area of 0.13 mm², were examined. The number of cells located at the glomerulus was also determined and expressed as cells per glomerulus.

To better identify arterioles, immunohistochemistry of α-smooth muscle actin (which stains the muscular layer of vessels) was performed using 4-μm-thick paraffin-embedded renal sections. Sections were mounted on glass slides, deparaffinized, and rehydrated using

Histology and morphometry: Sections 3-μm thick were stained with periodic acid-Schiff (PAS) reaction. The extent of glomerulosclerosis was evaluated using a simplification of the procedure described by Fujihara et al. (11, 12) by attributing a score to each glomerulus according to the extent of sclerotic injury: 0 = intact glomeruli; 1 = lesions affecting 50% or less of the glomerular area; and 2 = lesions affecting 51–100% of the glomerular area. The glomerulosclerosis index (GSI) was calculated for each rat as the weighted average of all individual glomerular scores thus obtained, multiplied by 100. At least 70 glomeruli were examined for each rat.
standard techniques. Sections were then exposed to microwave irradiation and preincubated with 5% normal horse serum with avidin and biotin and horse serum solutions as mentioned above. Incubation with the primary antibody, a monoclonal mouse antibody (Sigma-Aldrich), was carried out overnight at 4°C in a humidified chamber. Detection was performed by an indirect avidin-biotin-peroxidase technique. Briefly, after being washed, the sections were incubated at room temperature with rat-adsorbed biotinylated anti-mouse (Vector Labs) for 45 min and then with avidin-biotin-horseradish peroxidase (Vector) for an additional 30 min. Sections were finally incubated with a freshly prepared substrate, consisting of diaminobenzidine and H2O2, and developed in a dark chamber. Negative control experiments were performed by omitting incubation with the primary antibody. The thickness of the wall of afferent arterioles was evaluated by measuring the width of the stained muscular layer of a circular section of an arteriole in the vicinity of the glomerular vascular pole. Afferent arterioles were identified as vessels with less endothelium compared with the muscular layer and by the presence of elastic lamina. The quantification of vessel width was performed using image-analysis software (Image-Pro Plus) after-image acquisition using a digital system (Magnafire) coupled to an optical microscope (Olympus BX-50, Olympus optical, Tokyo, Japan).

Quantification of vessel width was performed using image-analysis software (Image-Pro Plus) after-image acquisition using a digital system (Magnafire) coupled to an optical microscope (Olympus BX-50, Olympus optical, Tokyo, Japan).

Given a normal distribution, Student’s unpaired t-test was employed employing the Kolmogorov-Smirnov test. Statistical analyses were performed using Student’s unpaired t-test with Welch’s correction, when appropriate. Statistical significance was defined as P < 0.05. All data are expressed as means ± SE.

RESULTS

Dietary administration of 0.3% I3C for 10 days resulted in the development of hypertension (173 ± 9 vs. 112 ± 11 mmHg, *P* < 0.01; Fig. 1A). As shown in Fig. 1B, the hypertensive rats also had a substantially lower body weight than the noninduced normotensive rats (218 ± 2 vs. 354 ± 6 g, *P* < 0.05). In this regard, and as previously observed (10–13), the rats induced with 0.3% I3C exhibited a marked reduction of body weight from 322 ± 7 to 218 ± 2 g (*P* < 0.05). The hypertensive animals also exhibited severe lethargy, piloerection, and assumption of a hunched posture, which are clinical manifestations of malignant hypertension in the rodent (23, 47, 48). Thus, as previously observed (27, 28, 31, 32), dietary administration of 0.3% I3C induced malignant hypertension in Cyp1a1-Ren2 rats.

As shown in Fig. 2 the general pathological view of the kidneys obtained from hypertensive Cyp1a1-Ren2 rats induced with I3C showed scattered vessels with concentric proliferative arteriolosclerosis, characteristic of malignant hypertension, as well as focal areas of tubule dilation. Glomerular damage was evaluated by determination of the GSI in tissue sections stained with PAS. Kidneys of hypertensive rats had a markedly higher GSI than normotensive rats (21.3 ± 5.6 vs. 3.5 ± 1.31 units, *P* < 0.05). Mesangial expansion, a characteristic feature of glomerulosclerosis, was observed in the hypertensive rats. Accordingly, the hypertensive rats had a mesangial area occupying 46 ± 3% of glomerular area compared with 10 ± 5% in normotensive rats (*P* < 0.05). Figure 2D shows a glomerulus with increased mesangial matrix (induced rat) opposed to a normal glomerulus illustrated in Fig. 2C (noninduced rat). Afferent arteriolar wall thickness averaged 5.95 ± 0.16 μm in the noninduced normotensive rats and was significantly increased in the induced hypertensive rats (6.65 ± 0.12 μm, *P* < 0.005).

Rats induced with I3C had marked tubulointerstitial inflammation characterized by infiltration of renal tissue by macrophages, as can be observed in Fig. 3B in contrast to normal kidney from noninduced rats shown in Fig. 3A. Immunohistochemical analysis of macrophage ED-1-positive cells following 10 days of induction of hypertension is presented in Fig. 5. Quantitative evaluation of macrophage ED-1-positive cells demonstrated infiltration of the renal cortical interstitium, where the vast majority of macrophages were found. In this anatomic site, there were 106.4 ± 38.2 macrophages/mm2 in the hypertensive rats, a value greater than in the normotensive rats (58.7 ± 5.0 macrophages/mm2, *P* < 0.01) (Figs. 3 and 5D). It was also observed that infiltrating macrophages clustered primarily in areas surrounding cortical arteries and arterioles (Fig. 3B). However, the number of macrophages infiltrating glomeruli did not differ between the hypertensive (1.4 ± 0.1 cells/glomerulus) and normotensive rats (1.4 ± 0.2 cells/glomerulus) (Figs. 3 and 5F).

To evaluate the presence of proliferating cells, immunohistochemistry was performed to stain cells containing PCNA. As shown in Fig. 4, the predominant location of PCNA-positive cells was again the interstitial area, with only a minority located in the renal vessels and glomeruli. Analysis of renal PCNA expression showed that cell proliferation was higher in...
tubules of hypertensive rats compared with normotensive rats (37.8 ± 5.7 vs. 24.2 ± 2.1 cells/mm², P < 0.05) and was particularly observed in dilated tubules. These results are shown qualitatively and quantitatively in Figs. 4, C and D, and 5B, respectively. Similarly, and as depicted in Fig. 5, A and C, there was also an increased number of proliferating cells in the cortical interstitium (33.6 ± 5.7 vs. 4.2 ± 1.4 cells/mm², P < 0.01), as well as in the walls of preglomerular vessels (2.2 ± 0.5 vs. 0.13 ± 0.07 cells/vessel, P < 0.01) of the induced rats. Representative pictures of interstitial proliferation can be seen in Fig. 4, C (noninduced rats) and E (rats induced with I3C), and pictures illustrating vessel wall proliferation are shown in Fig. 4, F (noninduced rats) and G (rats induced with I3C). However, the number of proliferating cells inside the glomeruli was not increased in the hypertensive rats (0.4 ± 0.1 vs. 0.3 ± 0.1 cells/glomerulus, not significant) (Fig. 5E). A representative picture demonstrating the lack of glomerular cell proliferation can be seen in Fig. 4, A (noninduced, normotensive rats) and B (induced, hypertensive rats).

**DISCUSSION**

The present study evaluated the renal morphological changes occurring during the development of ANG II-dependent malignant hypertension in Cyp1a1-Ren2 transgenic rats. In a previous study (23), it was demonstrated that Cyp1a1-Ren2 rats induced with 0.3% I3C for 14 days exhibited malignant vascular injury with fibrinoid necrosis and endarteritis of interlobular arterioles and afferent arterioles. In contrast, no afferent fibrinoid necrosis or endarteritis was observed in the kidneys of Cyp1a1-Ren2 rats induced with 0.3% I3C for 7 days (23). However, medial thickening of the vessel walls of interlobular and arcuate arteries was found by day 7 of induction (23). Consistent with these previous findings, in the present study we observed that Cyp1a1-Ren2 rats induced with 0.3% I3C for 10 days did not exhibit fibrinoid necrosis and endarteritis of the renal vasculature. Rather, the renal pathological changes observed included myointimal hyperplasia and tubular dilation, glomerulosclerosis, and tubulointerstitial inflammation and proliferation, particularly in the perivascular areas. Thus the results of the present study demonstrate that the renal pathological changes that occur 10 days after induction of malignant hypertension in Cyp1a1-Ren2 rats primarily consist of inflammation and cellular proliferation in the cortical vessels and tubulointerstitium. Such morphological changes together with preglomerular vasoconstriction may act to protect against transmission of the elevated arterial pressure to the glomeruli and thereby contribute to the maintenance of relatively normal values for glomerular filtration rate and renal plasma flow observed at this stage of the hypertension (31, 32). Presumably, continued exposure to the markedly elevated arterial pressure would result in the progression of more severe hypertensive vascular damage characteristic of malignant hypertension. One would predict that this would result in a marked decline in renal hemodynamic function; however, further studies are required to address this issue.

There is growing awareness that hypertension in general and hypertension combined with increased ANG II levels in particular are associated with inflammation and proliferative activity in renal tissue (4, 8, 11, 12, 17, 36, 39). Indeed, ANG II is a well-known proinflammatory agent that stimulates the synthesis of numerous cytokines (41), transcription factors (40), chemokines (30), and adhesion molecules (34). In addition, evidence obtained from numerous studies indicates that ANG II plays an important role in the pathophysiology of
several primarily nonimmunological renal disorders associated with renal inflammation such as diabetes, ureteral obstruction, and renal ablation (12, 15, 45). Furthermore, renal inflammation has been described in a variety of hypertensive models, including spontaneously hypertensive rats (SHR), ANG II-infused hypertensive rats, Dahl salt-sensitive rats, DOCA-salt hypertensive rats, and renin and angiotensinogen TGR[mRen2]27 double transgenic hypertensive rats (4, 8, 17, 36, 39). Similarly, hypertension resulting from chronic nitric oxide synthesis blockade and renal ablation has been shown to be associated with renal inflammation (11, 12). In some cases, renal interstitial inflammation has been found to occur before the development of hypertension as has been demonstrated in the SHR (37), suggesting that renal cortical interstitial inflammation per se may lead to hypertension. Interestingly, renal inflammation even in the embryo may determine later development of hypertension, as was demonstrated in a model where female pregnant rats were fed a low-protein diet (44). The previous demonstration that the immunosuppressant mycophenolate mofetil attenuates the severity of hypertension induced by chronic nitric oxide synthesis blockade (11, 16) and prevents the development of salt-sensitive hypertension that occurs following transient induction of ANG II-dependent hypertension (36) is indicative of an important role of renal inflammation in the development of hypertension. In the present study, macrophage infiltration occurred primarily in the perivascular areas and in the tubulointerstitial environment, suggesting that the perivascular interstitium as well as the peritubular interstitium are the regions where the heightened blood pressure initially transmits its harmful effects to renal tissue. It has often been postulated that mechanical forces caused by the elevated arterial pressures directly cause baromechanical trauma to renal tissue. Thus, as has been suggested for other hypertensive states (2), it is conceivable that barotrauma transmitted to the renal interstitium is a primary trigger of the renal inflammatory response occurring during the development of malignant hypertension in Cyp1a1-Ren2 transgenic rats. Further studies are required to determine the arterial pressure-dependent and -independent mechanisms underlying the renal injury occurring in ANG II-dependent malignant hypertension.

In the present study, we did not observe either inflammation or cellular infiltration of glomeruli despite pronounced mesangial enlargement. It is tempting to speculate that two different mechanisms are operating here. Namely, this would mean that in the perivascular area the elevated blood pressure is transmitted to the interstitium and triggers macrophage infiltration and cell proliferation, whereas glomeruli situated downstream of narrowed arterioles may have expansion of the mesangium in response to an ischemic environment. To the extent that this is the case, ischemic insult should determine glomerular damage characterized predominantly by matrix deposition compared with cellular infiltration. Additionally, high ANG II levels may contribute directly to the mesangial expansion as enlargement of mesangial area is frequently observed in other high-ANG II models of hypertension, such as ANG II-infused hypertensive rats (50), two-kidney, one-clip Goldblatt hypertensive rats (24), and the TGR(mRen2)27 transgenic rat (5, 6). In accordance with the morphological observations, and as mentioned above, physiological studies have shown that glomerular filtration rate and renal plasma flow are either normal or slightly reduced at this stage of the hypertension in Cyp1a1-Ren2 transgenic rats, suggesting that preglomerular vasoconstriction protects against transmission of the elevated arterial pressure to the glomeruli (27, 28, 31, 32). This is in accordance with a very elegant study in which the introduction of a maneuver that inhibits renal autoregulation and allows the transmission of heightened blood pressure to the glomeruli and postglomerular circulation transforms benign hypertension into a malignant form (3). In addition, when autoregulation is compromised but hypertension is treated, this transformation is prevented (3). Moreover, studies employing the two-kidney, one-clip Goldblatt model show more inflammation in the nonclipped kidney than the clipped kidney (9, 17, 18). In apparent contrast, however, some investigators have observed glomerular inflammation and mild mesangial cell proliferation in rats receiving ANG II infusion (22, 40). These authors suggested that ischemia by itself may lead to renal inflammation, as has been shown in aortic coarctation, where the
constriction of the aorta above the emergence of the renal arteries prevents the kidneys from being exposed to the deleterious effects of the heightened blood pressure (38) but the kidneys exhibit inflammatory changes. Although the reason for these apparent discrepancies remains unclear, differences among the models, particularly regarding inhibition of renin release blockade by ANG II infusion, may account for the differences observed.

In contrast to the glomeruli, which exhibited little if any inflammatory changes, the abnormalities in the tubular and interstitial area were much more pronounced in Cyp1a1-Ren2 rats with malignant hypertension. While the presence of mac-

Fig. 4. Immunohistochemistry of proliferating cells (nuclei in red). A: glomerulus of Cyp1a1-Ren2 TGR receiving normal rat chow for 10 days. B: glomerulus of Cyp1a1-Ren2 TGR induced with 0.3% I3C for 10 days. C: normal renal tissue (smaller magnification). D: dilated cortical tubules in Cyp1a1-Ren2 rats induced with 0.3% I3C for 10 days. E: perivascular area in Cyp1a1-Ren2 rats induced with 0.3% I3C for 10 days. F: normal renal artery. G: vascular wall in rats induced with 0.3% I3C for 10 days.
raphages and proliferating cells in the perivascular sites may be explained as a consequence of the heightened blood pressure inside the vessel, the marked increase in the number of PCNA-positive cells in areas of tubule dilation requires another pathophysiological explanation. In this regard, it is possible that the enhanced PCNA cell number observed in the rats with malignant hypertension reflected increased tubular cell proliferation secondary to tubular dilation induced by glomerular ischemia. In essence, it is possible that glomerular ischemia resulted in tubular cell atrophy and dilation and that the increased PCNA cell number reflected the increased cell proliferation that occurred to repopulate the damaged tubules (1). Further studies are required to address this issue.

Regardless of the specific mechanisms responsible for the development of renal interstitial inflammation in the Cyp1a1-Ren2 transgenic rats with malignant hypertension, one would predict that the renal interstitial inflammation combined would contribute importantly to the maintenance of malignant hypertension as well as to the hypertensive damage itself. In this regard, biochemical mediators of inflammation are known to play a role in sodium retention and in renal vasoconstriction observed in hypertension. The most extensively studied candidates for translating renal inflammation into hypertension are locally produced ANG II and reactive oxygen species (16, 19, 25, 32, 42, 46, 49). Both are released in renal inflammation and possess well-known renal actions determining renal sodium retention and hypertension. Indeed, it has been described that renal damage in hypertension is associated with intra-renal production of ANG II independently of systemic activation of the renin-angiotensin system (2, 16) and that blockade of AT1 receptors even without decreasing blood pressure is capable of preventing renal damage in a malignant hypertension model (42). Similarly, reactive oxygen species production is increased in renal inflammation and is involved in development of hypertension (19, 25, 32, 46, 49). Moreover, a variety of inflammatory mediators, such as NF-κB and Rho-kinase, is involved in renal damage in hypertension, as the inhibition of NF-κB ameliorates renal damage in the double-transgenic (dTGR) rat harboring both human renin and angiotensinogen genes (29) and the long-term administration of Rho-kinase inhibitor fasudil ameliorates renal damage in a model of malignant hypertension (20). Thus the results of the present study are consistent with these previous findings and indicate that renal inflammatory changes occur during and contribute to the development of ANG II-dependent malignant hypertension in Cyp1a1-Ren2 transgenic rats.

In summary, the results of the present study demonstrate that the renal pathological changes that occur during the develop-
ACKNOWLEDGMENTS

The authors thank Drs. Ron Rose and Barb Mickelson of Harlan-Teklad for assistance with the design and production of the IEC-containing rat diet.

GRANTS

This study was supported by grants from the American Heart Association, NHLBI grant HL26371, and the Louisiana Board of Regents Millennium Trust Health Excellence Fund (2001-06-07). Portions of this work were presented in abstract form at the Experimental Biology 2005 Meeting in San Diego, CA (FASEB J 19: A548, 2005. M. L. Graciano was the recipient of a National Kidney Foundation Post Doctoral Fellowship Award for the duration of this study.

REFERENCES


