Urokinase-Type Plasminogen Activator Plays a Critical Role in Angiotensin II–Induced Abdominal Aortic Aneurysm

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Abstract—We have previously demonstrated that urokinase-type plasminogen activator (uPA) is highly expressed in the aneurysmal segment of the abdominal aorta (AAA) in apolipoprotein E–deficient (apoE−/−) mice treated with angiotensin II (Ang II). In the present study, we tested the hypothesis that uPA is essential for AAA formation in this model. An osmotic minipump containing Ang II (1.44 mg/kg per day) was implanted subcutaneously into 7- to 11-month-old male mice for 1 month. Ang II induced AAA in 7 (90%) of 10 hyperlipidemic mice deficient in apoE (apoE−/−/uPA−/− mice) but in only 2 (22%) of 9 mice deficient in both apoE and uPA (apoE−/−/uPA+/− mice) (P<0.05). Although the expansion of the suprarenal aorta was significantly less in apoE−/−/uPA−/− mice than in apoE−/−/uPA+/− mice, the aortic diameters of the aorta immediately above or below the suprarenal aorta were similar between the 2 groups. Ang II induced AAA in 18 (39%) of 46 strain-matched wild-type C57 black/6J control mice. The incidence was significantly higher in atherosclerotic apoE-deficient (apoE−/−) mice, in which 8 (100%) of 8 mice developed AAA. Only 1 (4%) of 27 uPA−/− mice developed AAA after Ang II treatment. We conclude the following: (1) uPA plays an essential role in Ang II–induced AAA in mice with or without preexisting hyperlipidemia and atherosclerosis; (2) uPA deficiency does not affect the diameter of the nonaneurysmal portion of the aorta; and (3) atherosclerosis and/or hyperlipidemia promotes but is not essential for Ang II–induced AAA formation in this model. (Circ Res. 2003;92:510-517.)

Key Words: urokinase-type plasminogen activator deficiency • aneurysm • atherosclerosis • vascular inflammation

Atherosclerosis is an inflammatory disease characterized by the infiltration of monocytes/macrophages and lymphocytes into the arterial wall.1,2 Vascular inflammation is also a prominent feature of atherosclerotic abdominal aortic aneurysms (AAAs).3–5 Monocytes/macrophages are a major source of urokinase-type plasminogen activator (uPA) in atherosclerotic lesions.6,7 UPA hydrolyzes plasminogen to form plasmin, a trypsin-like proteolytic enzyme capable of directly degrading components of the extracellular matrix as well as activating matrix metalloproteinases (MMPs).6,8,9 MMPs are known to degrade the extracellular matrix, including collagen and elastin, thus impairing the structural integrity of the vascular wall and contributing to AAA formation.10 Atherosclerotic lesions in the aorta of high cholesterol diet–fed apolipoprotein E–deficient (apoE−/−) mice show fragmentation of the elastic lamellae and rupture of the media, resulting in aneurysm formation. These pathological changes were not observed in mice deficient in both apoE and uPA (apoE−/−/uPA−/− mice), suggesting that uPA may play an important role in matrix destruction and aneurysm formation.8,11 Chronic infusion of angiotensin II (Ang II) has been shown to induce AAA in apoE−/− mice.12 We demonstrated that uPA is highly expressed in the aneurysmal segment of the abdominal aorta in this model.13 The present study was designed to test the hypothesis that uPA plays a critical role in Ang II–induced AAA formation in mice. Our finding that uPA deficiency reduces the incidence and complexity of Ang II–induced AAA in C57 black/6J and apoE−/− mice validates the above hypothesis.

Materials and Methods

Animal Preparation

All experimental procedures were approved by the Institutional Animal Care and Use Committee and were in accordance with the Guide for the Care and Use of Laboratory Animals (NIH). Osmotic minipumps (model 2004, Alzet) containing Ang II (1.44 mg/kg per day) were implanted subcutaneously into 7- to 11-month-old male mice. The following groups were studied: (1) apolipoprotein E–deficient mice with a C57 black/6J background (apoE−/− mice) and strain-matched wild-type C57 black/6J mice (control); (2) uPA–deficient mice with a C57 black/6J background (uPA−/− mice) and
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strain-matched wild-type C57 black/6J control mice; and (3) apoE-uPA double-deficient mice (apoE<sup>−/−</sup>/uPA<sup>−/−</sup>) and littermate control mice with an apoE single deficiency (apoE<sup>−/−</sup>/uPA<sup>+/+</sup>) mice. The parent apoE<sup>−/−</sup>/uPA<sup>+/+</sup> and apoE<sup>−/−</sup>/uPA<sup>−/−</sup> mice were obtained from the University of Leuven, Leuven, Belgium. The other mice were obtained from Jackson Laboratories (Bar Harbor, Maine). At the end of 1 month of treatment with Ang II, blood pressure was measured noninvasively by the tail-cuff method. Before the mice were euthanized, blood samples were obtained for the measurement of serum cholesterol and triglyceride levels (Consolidated Veterinary Diagnostics). The animals were then euthanized, and the abdominal aorta was examined for the presence of AAA.

### Measurement of Diameters of Suprarenal Aorta

In the C57 black/6J mice, the cross-sectional diameters of the suprarenal aorta were measured as described previously. After the animals were euthanized, the aortas were perfused at a constant pressure of 100 mm Hg through the heart with PBS, followed by warm (37°C) agarose (SeaPlaque GTG Agarose, low-melt, FMC BioProducts) diluted in saline (3% wt/vol) and colored with green tissue dye. After the agarose had solidified, the abdominal aorta was dissected free from the surrounding connective tissue and pinned down to a wax block before fixation in 10% formalin. Cross sections of the aorta (2.5 mm in thickness) were made between the superior mesenteric and right renal arteries. A small portion of the right renal artery was left attached to the samples to facilitate orientation of the specimen. These tissues were dehydrated through a graded ethanol series, cleared with xylene, infiltrated with warm paraffin, embedded in paraffin blocks, cut at 5-μm thickness, and stained with hematoxylin and eosin. The lumen and adventitial circumferences at the maximal expanded portion of the suprarenal aorta were quantified by CtrSimple Imaging Systems (Compu); this quantification was then used to calculate the luminal and outer diameters of the vessel. The wall thickness was calculated from the difference between the luminal and outer diameters.

A direct method was also used to measure the outer diameter of the suprarenal aorta. After the aorta was dissected free from the surrounding connective tissue, a picture was taken with a digital camera. The image of the aorta was used to measure the outer diameter of the suprarenal aorta. In some groups, the outer diameters of the thoracic aorta immediately above the diaphragm and the abdominal aorta immediately below the right renal artery were also measured using the same method. This direct measurement was compared with the measurement of the cross section as described above. The results were very similar between the 2 methods. Therefore, in all the other groups of mice, except for some of the C57 black/6J mice, only the direct method was used to measure the outer diameter of the suprarenal aorta. On the basis of this direct measurement, we set a threshold of 1.22 mm for suprarenal aortic diameter as a criterion for calculating the incidence of aneurysm. This criterion is based on the fact that the average diameter of suprarenal aorta in control mice not treated with Ang II is 0.8 to 1.0 mm. An increase in the suprarenal aorta to 1.22 mm is 50% expansion.

### Classification of Aneurysm

A 4-point grading system was used to classify aneurysms as described in detail by Daugherty et al: type 0, no aneurysm; type I, dilated lumen in the suprarenal region of the aorta with no thrombus; type II, remodeling tissue in the suprarenal region that frequently contained thrombus; type III, pronounced bulbous form of type II that contained thrombus; and type IV, a form in which there are multiple aneurysms containing thrombus. No animals were found to have type IV aneurysm in the present study.

### Quantification of Atherosclerotic Plaques in the Carotid Arteries

The left and right carotid arteries were dissected, cut open longitudinally, and pinned down individually on silicon-coated Petri dishes. Atherosclerotic plaques were visible without staining. Images of the open luminal surface of the vessels were captured with a digital camera mounted on a dissecting microscope. The plaque area was quantified using a C-Simple system (Compu) and expressed as a percentage of the total luminal surface area as described in detail previously.

### In Situ Hybridization

The suprarenal aortas from Ang II–treated C57 black/6J mice that developed AAA were immersed in a fixative solution containing 1 part zinc formalin (Anatech) with 4 parts DEPC water for 4 hours. After the fixative solution was removed, the tissue samples were immersed overnight in 70% ethanol diluted with DEPC water. The tissues were analyzed by in situ hybridization for uPA and plasminogen activator inhibitor (PAI)-1 using 35S-labeled cRNA probes. Both the sense and antisense probes were labeled to specific activities between 0.5×10⁶ cpm/mg RNA and 2×10⁷ cpm/mg RNA. The exposure time was 6 weeks. In all experiments, an 35S-labeled sense control probe was hybridized to adjacent tissue sections on the same slide. Results using this sense control were uniformly negative.

### Zymography

Protein extracts were prepared from suprarenal aortas and aortic arches from Ang II–treated C57 black/6J mice that developed AAA. In a separate experiment, protein extracts were also prepared from the suprarenal aortas from an apoE<sup>−/−</sup>/uPA<sup>−/−</sup> mouse with no AAA and from an apoE<sup>−/−</sup>/uPA<sup>++</sup> mouse that developed AAA. Samples were resolved under nonreducing condition on a 10% polyacrylamide gel containing 0.1% gelatin as the substrate for MMP activity. After electrophoresis, proteins were renatured by a wash in Triton X-100. The gels were then incubated overnight at 37°C in a solution containing 50 mmol/L Tris, 200 mmol/L NaCl, and 5 mmol/L CaCl₂, pH 8.0. Zones of lysis were visualized by staining with 0.5% Coomassie blue R-250.

### Ex Vivo Aortic Organ Culture and IL-6 Assay

The aortas from C57 black/6J mice treated with vehicle or Ang II (only those that developed AAA) were dissected and cut into 3 segments: the aortic arch, suprarenal aorta, and the rest of the abdominal aorta. The fresh aortic segments were immediately placed in 1 mL DMEM medium (GIBCO-BRL) containing 1× ITS (containing insulin, transferrin, and selenium, GIBCO-BRL) and 0.1% BSA (Sigma Chemical Co). The secretion of interleukin (IL)-6 was measured using a commercially available murine IL-6 ELISA kit (Biokines, Amersham Life Sciences) as described in detail previously. The IL-6 values were normalized to the weight of the aortic tissue segment and expressed as picograms per milliliter per milligram tissue.

### Noninvasive Measurement of Systolic Blood Pressure

Systolic blood pressure was measured in conscious uPA<sup>−/−</sup>, apoE<sup>−/−</sup>, and C57 black/6J mice using the tail-cuff system (Kent Scientific). The mice were trained to stay quietly in a restrainer placed on a warm pad for a period of at least 30 minutes for 1 to 4 days before the study. On the day of the study, the mice were placed in the temperature-controlled restrainer for 15 minutes. Blood pressure was then measured repeatedly and recorded on a data acquisition system (PowerLab, 16/8, ADInstruments). Systolic blood pressure was averaged from 5 consecutive measurements.

### Statistical Analysis

Results are presented as mean±SEM for the number of animals (n) indicated. Multiple comparisons of the mean values were performed by ANOVA, followed, if significance was indicated, by a subsequent Student-Newman-Keuls test for repeated comparisons. The percentage of mice that developed AAA was compared between 2 groups by use of the χ² test. Differences were considered to be statistically significant at P<0.05, Statistica software (StatSoft, Inc) was used for the statistical analysis.
Results

Effects of Ang II in Wild-Type C57 Black/6J Mice Compared With Atherosclerotic apoE<sup>−/−</sup> Mice

Infusion of Ang II induced AAA formation in 7 (39%) of 18 wild-type C57 black/6J mice. The incidence of AAA was significantly lower than that in atherosclerotic apoE<sup>−/−</sup> mice, in which 8 (100%) of 8 mice developed AAA (Figure 1). Consequently, the average diameter of the suprarenal aorta was significantly smaller in C57 black/6J mice than in apoE<sup>−/−</sup> mice (Figure 1). In the 7 C57 black/6J mice that developed AAA, the average diameter of the suprarenal aorta (2.1 ± 0.2 mm) was not significantly different from that in the apoE<sup>−/−</sup> mice (1.9 ± 0.2 mm). As expected, atherosclerotic lesions were observed by en face examination of the aorta of the apoE<sup>−/−</sup> but in none of the C57 black/6J control mice. The AAAs that developed in both the C57 black/6J and apoE<sup>−/−</sup> mice were all consistently localized to the suprarenal aorta and were characterized by an expansion of both the luminal and outer diameters as well as an increase in wall thickness (Figure 2). Blood pressure measured 1 month after Ang II treatment was not significantly different between the apoE<sup>−/−</sup> mice (142 ± 5 mm Hg, n = 13) and C57 black/6J control mice (156 ± 7 mm Hg, n = 22). As expected, the serum cholesterol level was significantly higher in apoE<sup>−/−</sup> mice (615 ± 35 mg/mL) than in C57 black/6J control mice (166 ± 8 mg/mL).

Expression of uPA and MMPs and Secretion of IL-6 in the Aortas of C57 Black/6J Mice Treated With Ang II

A strong signal for uPA was detected by in situ hybridization of the aneurysmal section of the suprarenal aorta isolated from C57 black/6J mice. The uPA signal was colocalized with infiltrating mononuclear cells (Figure 3). The signal for PAI-1 mRNA was also detected in mononuclear cells in the inflammatory infiltrates as well, although it was weaker than the signal for uPA (Figure 3). In Ang II–treated C57 black/6J mice that did not develop AAA, no uPA signal was detected in the suprarenal aorta (data not shown). However, a PAI-1 signal, colocalized with smooth muscle cells, was detected.

Gelatin zymography of tissue extracts detected both pro and activated forms of MMP-2 in the aneurysmal section of the suprarenal aorta as well as in the aortic arch of C57 black/6J mice (Figure 4). However, MMP-9 activity was predominantly detected only in the aneurysmal tissue. The same image from the lane of the aortic arch of this gel has been used in a previous publication for comparison with apoE<sup>−/−</sup> mice, showing that both MMP-2 and MMP-9 were highly expressed in aneurysmal tissue as well as in the nonaneurysmal atherosclerotic aortic arch.13

Ex vivo secretion of IL-6 protein was also significantly increased in all sections of the freshly isolated aorta of C57
black/6J mice treated with Ang II compared with those treated with vehicle (Figure 5).

**Effects of Ang II in Nonatherosclerotic uPA−/− Mice Compared With Wild-Type C57 Black/6J Control Mice**

Compared with C57 black/6J mice, the incidence of Ang II–induced AAA was significantly reduced in uPA−/− mice (Figure 6). Correspondingly, the average outer diameter of the suprarenal aorta was significantly smaller in uPA−/− mice than in C57 black/6J control mice treated with Ang II. No atherosclerotic lesions were observed in Ang II–treated uPA−/− or C57 black/6J mice. Systolic blood pressure was not significantly different between the uPA−/− mice (155±11 mm Hg, n=6) and C57 black/6J control mice (156±7 mm Hg, n=22) after treatment with Ang II for 1 month.

**Effects of Ang II in Atherosclerotic ApoE−/−/uPA−/− Mice Compared With ApoE−/−/uPA+/+ Mice**

Infusion of Ang II induced AAA formation in 9 (90%) of 10 apoE−/−/uPA−/− mice. The incidence of AAA was significantly reduced to 2 (22%) of 9 apoE−/−/uPA−/− mice (Figure 7, top). Correspondingly, the outer diameters of the suprare-
nal aortas were significantly smaller in apoE<sup>−/−</sup>/uPA<sup>−/−</sup> mice compared with apoE<sup>−/−</sup>/uPA<sup>−/−</sup> mice (Figure 7, middle). For apoE<sup>−/−</sup>/uPA<sup>−/−</sup> mice compared with apoE<sup>−/−</sup>/uPA<sup>−/−</sup> mice, the diameters of the thoracic aortas (1.04 ± 0.10 versus 1.08 ± 0.05 mm, respectively) and the infrarenal aortas (0.72 ± 0.03 versus 0.86 ± 0.06 mm, respectively) were not significantly different between 2 groups. The complexity of the various AAAs in these mice is summarized in Figure 7 (bottom). Although the apoE<sup>−/−</sup>/uPA<sup>−/−</sup> mice developed a number of type II or III aneurysms, the aneurysms that developed in only 2 of the apoE<sup>−/−</sup>/uPA<sup>−/−</sup> mice were type I (small and simple dilatation of the suprarenal aorta). For Ang II–treated apoE<sup>−/−</sup>/uPA<sup>−/−</sup> mice and apoE<sup>−/−</sup>/uPA<sup>−/−</sup> mice, serum cholesterol levels (560 ± 42 versus 564 ± 46 mg/mL, respectively) and atherosclerotic plaque areas in the carotid artery (26 ± 3% versus 22 ± 5%, respectively) were not significantly different.

Zymographic analysis of MMP activities in the suprarenal aortas did not show significant differences in pro-MMP-2 and MMP-9 activities between the apoE<sup>−/−</sup>/uPA<sup>−/−</sup> and apoE<sup>−/−</sup>/uPA<sup>−/−</sup> mice, whereas the activated MMP-2 activity appeared to be decreased slightly in apoE<sup>−/−</sup>/uPA<sup>−/−</sup> mice (Figure 8).

**Discussion**

**Atherosclerosis Potentiates but Is Not Essential for Ang II–Induced Aneurysm Formation**

In the present study, chronic infusion of Ang II for 1 month induced AAA in 39% of wild-type C57 black/6J mice, indicating that apoE deficiency with preexisting hyperlipidemia and/or atherosclerosis is not essential for AAA formation in this model. Because Ang II induced AAA in almost all of the apoE<sup>−/−</sup> mice, apoE deficiency with preexisting atherosclerosis and/or hypercholesteremia apparently potentiates Ang II–induced AAA formation. We have reported previously that systolic blood pressure in mice without treatment of Ang II is ~120 mm Hg. Treatment with Ang II modestly increased the blood pressure, raising the possibility that hypertension may contribute to AAA formation. However, because blood pressure was not significantly different between the 2 groups after Ang II treatment, the increased incidence of AAA in apoE<sup>−/−</sup> mice compared with that in C57 black/6J control mice cannot be explained by hemodynamic changes. In contrast to our results, Daugherty et al. did not detect a significant change in blood pressure by Ang II in the same model in female mice. It has been demonstrated that the...
vasopressor response in females could be much lower than that in males. Another possible reason could be that we measured blood pressure in conscious animals rather than in anesthetized ones.

Characteristically, the pathological changes in the suprarenal aortic wall of the C57 black/6J mice that developed AAA were similar to those of the apoE<sup>−/−</sup> mice. Both showed vascular inflammation with macrophage infiltration, vascular wall thickening, and expansion of the luminal and outer diameters of the suprarenal aorta. The proinflammatory, pro-oxidant, and proliferative actions of Ang II in the vascular wall most likely mediate AAA formation. Ang II promotes recruitment of inflammatory cells into the vessel wall by inducing the expression of monocyte chemoattractant protein-1. Ang II also increases low density lipoprotein oxidation, macrophage 12/15-lipoxygenase activity, and IL-6 secretion. IL-6 is a marker of vascular inflammation. In human AAA, the circulating level of IL-6 is elevated and positively correlated with aortic diameter expansion. Consistent with these reports, chronic treatment with Ang II increased IL-6 secretion from the aortas of apoE<sup>−/−</sup> mice and wild-type C57 black/6J control mice, as shown in the present study. Monocytes/macrophages are a major source of proteolytic enzymes, such as uPA and MMPs. These proteinases can degrade the major structural proteins in the vessel wall, thus contributing to AAA formation.

In atherosclerotic apoE<sup>−/−</sup> proteins in the vessel wall, thus contributing to AAA forma-

tion. In atherosclerotic apoE<sup>−/−</sup> mice, abundant inflammatory cells, including monocytes, macrophages, and foam cells, are present in the aortic wall. The chemoattrant and proinflam-
matory effects of Ang II can further augment vascular inflammation, thus potentiating atherosclerosis and AAA formation. It has been reported that apoE may possess direct anti-inflammatory and antiatherogenic effects. Compromise of these functions in apoE<sup>−/−</sup> mice may also contribute to the greater vascular inflammation, atherosclerosis, and AAA formation observed in the present study.

In the apoE<sup>−/−</sup> mice treated with Ang II, both MMP-2 and MMP-9 were highly expressed in atherosclerotic tissue as well as in the nonaneurysmal atherosclerotic aortic arch. In Ang II–treated C57 black/6J mice that developed AAA, MMP-2 was highly expressed in both the aortic arch and aneurysm, whereas MMP-9 expression was localized only to the aeur-
ysm. MMP-2 is mainly expressed in smooth muscle cells and adventitial fibroblasts, whereas MMP-9 is expressed predominantly by macrophages but also by smooth muscle. The atherosclerosis-rich aortic arch of the apoE<sup>−/−</sup> mice was infiltrated with macrophages, whereas there were no atherosclerotic lesions in Ang II–treated C57 black/6J mice. The localization of macrophages to the aneurysm of C57 black/6J mice explains the selective expression of MMP-9 in the C57 black/6J mice compared with the more extensive expression of MMP-9 in the atherosclerotic aorta of the apoE<sup>−/−</sup> mice. The identification of both MMP-2 and MMP-9 in this mouse model of AAA is correlated with the expression of these elastolytic enzymes in human AAA tissue.

uPA Plays a Critical Role in Ang II–Induced Aneurysm Formation

It has been reported that the pathogenesis of atherosclerotic AAA formation involves vascular wall proteolysis induced by activation of the plasmin-MMP system. uPA and its receptors promote vascular inflammation by acting as a potent chemotactant and by activating cytokines, which may further facilitate macrophage infiltration into the vascular wall. These inflammatory cells are a major source of uPA and MMPs. It has been observed that mice deficient in uPA have decreased inflammatory cell infiltration at the sites of vascular injury. In a previous study, we demonstrated that uPA is highly expressed in the aneurysmal segment of the abdominal aorta of Ang II–treated apoE<sup>−/−</sup> mice. Increased expression of uPA has also been reported in human AAA. In the present study, in situ hybridization detected uPA associated with infiltrating monocytes in atherosclerotic lesions induced by Ang II. Such data demonstrated an association between uPA and AAA formation, but these data do not prove cause and effect. Our present results (ie, the incidence of AAA induced by Ang II was greatly reduced in uPA<sup>−/−</sup> mice) demonstrate that uPA plays a major causative role in AAA formation in this model. The attenuation of AAA in uPA mice cannot be attributed to hemodynamic factors, because blood pressure was not significantly different between C57 black/6J and uPA<sup>−/−</sup> mice after Ang II treatment.

It is known that the activity of uPA can be regulated by local concentrations of its major endogenous inhibitor, PAI-1. Increased PAI-1 expression has been reported in human AAA. Although the present in situ hybridization study also detected PAI-1 in the atherosclerotic tissue from the C57 black/6J mice treated with Ang II, the signal was relatively weak compared with that resulting from uPA. Furthermore, the present results also demonstrated that in animals with preexisting atherosclerosis, the incidence of AAA induced by Ang II was significantly lower in apoE<sup>−/−</sup>/uPA<sup>−/−</sup> mice compared with apoE<sup>−/−</sup>/uPA<sup>+/−</sup> littermate control mice. The atherosclerums that developed in only 2 of the 9 apoE<sup>−/−</sup>/uPA<sup>−/−</sup> mice were small type I atherosclerums. In con-
tast, the atherosclerums that developed in apoE<sup>−/−</sup>/uPA<sup>+/−</sup> mice showed more complex pathology, characterized by large expansion of the suprarenal aorta and thrombus formation (type II or III). Thus, uPA deficiency provided protection against Ang II–induced AAA development even in the presence of apoE deficiency and atherosclerosis. This protective effect of uPA deficiency occurred without changes in atherosclerotic plaque area and serum cholesterol levels. These results demonstrate for the first time that uPA plays an essential role in Ang II–induced AAA formation in both the presence and absence of hyperlipidemia and atherosclerosis. The fact that uPA deficiency does not affect the diameters of the nonaneurysmal portion of the aorta suggests that uPA is not required for normal vascular development and maintenance.

uPA deficiency is known to reduce cell migration into the vessel wall after injury. This could also be one of the underlying mechanisms for the reduced AAA formation in uPA-deficient mice, inasmuch as infiltration of inflammatory cells into the vessel wall is a major pathological event in the atherosclerotic wall in this model. On the other hand, uPA activity concentrated to the cell surface by the uPA receptor can activate plasminogen locally, and plasmin, in turn, degrades laminin, fibronectin, and fibrin. Vessel wall fibrin...
deposition has been seen in atherosclerotic apoE-/- mice, and it could form a barrier for migrating cells in apoE-/-/uPA-/- mice. The contribution of MMP activities to the formation of AAA in this model is not clear. Our previous data showed that increased MMP-2 and MMP-9 activities are associated with both the aortic arch and abdominal aorta in apoE-/- mice. MMP activities, unlike uPA, are not specific to the AAA tissues. In the present study, we observed that there were MMP-2 and MMP-9 activities in the nonaneurysmal abdominal aorta of apoE-/-/uPA-/- mice, suggesting that uPA is not crucial for MMP-2 and MMP-9 activation. In the suprarenal aortas, there appears a slight reduction in activated MMP-2 activity in apoE-/-/uPA-/- mice. Reduced MMP-2 activity could be due to uPA deficiency and could also contribute, at least in part, to decreased AAA formation in apoE-/-/uPA-/- mice.

In summary, the present study demonstrates that Ang II induces AAA in both apoE-/- and C57 black/6J mice. Genetic deficiency of uPA protects both apoE-/- and C57 black/6J mice from Ang II-induced AAA formation but does not affect the diameters of the nonaneurysmal portion of the aorta. These results indicate that uPA plays a critical role in Ang II-induced AAA in mice but is not required for normal vascular development and maintenance. The incidence of AAA induced by Ang II was greater in apoE-/- than in C57 black/6J mice, indicating that preexisting hyperlipidemia and atherosclerosis potentiate but are not essential for Ang II-induced AAA formation.

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