Macrophage-Targeted Overexpression of Urokinase Causes Accelerated Atherosclerosis, Coronary Artery Occlusions, and Premature Death

Aaron E. Cozen, BA; Hideaki Moriwaki, MD, PhD; Michal Kremen, MD; Mary Beth DeYoung, PhD; Helén L. Dichek, MD; Katherine I. Slezicki; Stephen G. Young, MD; Murielle Véniant, PhD; David A. Dichek, MD

Background—Human atherosclerotic lesions contain elevated levels of urokinase plasminogen activator (uPA), expressed predominantly by macrophages.

Methods and Results—To test the hypothesis that macrophage-expressed uPA contributes to the progression and complications of atherosclerosis, we generated transgenic mice with macrophage-targeted overexpression of uPA. The uPA transgene was bred into the apolipoprotein E–null background, and transgenic mice and nontransgenic littermate controls were fed an atherogenic diet. uPA-transgenic mice had significantly elevated uPA activity in the atherosclerotic artery wall, of a magnitude similar to elevations reported in atherosclerotic human arteries. Compared with littermate controls, uPA-transgenic mice had accelerated atherosclerosis, dilated aortic roots, occlusive proximal coronary artery disease, myocardial infarcts, and early mortality.

Conclusions—These data support the hypothesis that overexpression of uPA by artery wall macrophages is atherogenic and suggest that uPA inhibitors might be therapeutically useful. (Circulation. 2004;109:2129-2135.)

Key Words: urokinase ■ atherosclerosis ■ coronary disease

Urokinase plasminogen activator (uPA) is expressed by human arterial wall macrophages1–3 and may contribute to the pathogenesis of atherosclerosis. uPA expression is elevated in atherosclerotic human arteries, 2- to 5-fold above levels in normal arteries.1–4 Moreover, within individual coronary arteries, the level of local uPA expression is directly correlated with atherosclerosis severity.3 Elevated uPA expression may also be associated with complications of atherosclerosis including aneurysm formation and plaque rupture. uPA expression is increased in human aneurysms,5 and uPA acts via plasmin to activate collagenases,6 which are potential triggers of plaque rupture.7 However, few prospective experimental data support a causal link between uPA and atherosclerosis.

Studies in genetically modified mice8–13 do not, in general, support an atherogenic role for uPA. For example, apolipoprotein E–null (Apoe−/−) mice also deficient in uPA had the same amount of atherosclerosis as uPA-expressing controls.8 In 3 separate studies, Apoe−/− mice deficient in plasminogen activator inhibitor 1 (PAI-1; the primary physiological inhibitor of uPA) had either the same,10 less,11 or more atherosclerosis12 than wild-type mice. Apoe−/− mice deficient in plasminogen (the primary physiological substrate of uPA) had increased atherosclerosis,9 consistent with antiatherogenic roles for plasminogen and its activator, uPA. Apoe−/− mice with macrophage-specific overexpression of collagenase had decreased atherosclerosis.13 This study is relevant to uPA because collagenases are activated by the uPA/plasminogen system; therefore, the effect of elevated collagenase activity might predict the effect of elevated uPA. Decreased atherosclerosis in collagenase-overexpressing mice together with increased atherosclerosis in plasminogen-null mice supports a general hypothesis that elevated arterial wall proteolytic activity decreases atherosclerosis.14 This hypothesis predicts that elevated arterial uPA expression would be antiatherogenic.

Although many of these murine atherosclerosis studies predict an antiatherogenic role for uPA, virtually all of these studies involve manipulation of molecules other than uPA. In the single study in which uPA expression was manipulated directly, uPA expression was eliminated entirely.8 In contrast, uPA expression is increased in atherosclerotic human arteries.1–3 We hypothesized that uPA has atherogenic activity that is manifested when it is expressed at elevated levels by
macrophages. This hypothesis was supported by experiments in which gene transfer of uPA to the carotid arteries of hypercholesterolemic rabbits increased intimal growth.\textsuperscript{15} However, in this short-term study, uPA was expressed in endothelial cells, whereas macrophages are the primary cell type that expresses uPA in human lesions. To test the hypothesis that macrophage-expressed uPA is atherogenic, we generated transgenic mice with macrophage-targeted overexpression of uPA, bred the transgene into the \textit{Apoe} \textsuperscript{-/-} background, and evaluated the development of atherosclerosis.

**Methods**

**Generation of Transgenic Mice**

A macrophage-targeted uPA transgene was constructed by fusing promoter and enhancer sequences from the human scavenger receptor A gene (from C. Glass, University of California, San Diego)\textsuperscript{16} to the mouse uPA gene\textsuperscript{17} (from J. Degen, Children’s Hospital Medical Center, Cincinnati, Ohio). This “SR-uPA” transgene was used to generate transgenic mice in the C57BL/6 \textit{Apoe} \textsuperscript{-/-} background. All animal protocols were approved by the institutional animal care and use committees.

**Atherosclerosis Studies**

The SR-uPA transgene was established in the C57BL/6 \textit{Apoe} \textsuperscript{-/-} background by serial breedings with C57BL/6 \textit{Apoe} \textsuperscript{-/-} mice (Jackson Laboratories, Bar Harbor, Maine). SR-uPA \textit{Apoe} \textsuperscript{-/-} mice were identified by Southern analysis of tail-tip DNA and Western blotting of plasma to confirm the absence of apo E.\textsuperscript{18} Mice in the atherosclerosis studies were at least 94% C57BL/6 background. All atherosclerosis studies compare transgenic mice and nontransgenic littermates, which controls for the limited numbers (\textless;6%) of non-C57BL/6 genes. Female mice 5 weeks of age were begun on a diet containing 21% fat and 0.15% cholesterol by weight (TD88137; Harlan Teklad).

**Plasma Lipids**

Cholesterol and triglyceride levels were measured (Spectrum cholesterol assay, Abbott, and TG triglyceride kit, Boehringer Mannheim). Lipoproteins were fractionated by fast protein liquid chromatography (FPLC).\textsuperscript{19}

**Northern Analysis**

Organs were snap-frozen and stored at −80°C, and RNA was extracted with TRIzol (Gibco BRL). Macrophage RNA was obtained by applying TRIzol directly to the cultured cells. Blots were hybridized to uPA and GAPDH cDNA probes. Bound probes were quantified with a phosphorimager.\textsuperscript{15,20}

**Macrophage Plasminogen Activation**

Macrophages were collected by peritoneal lavage 3 days after intraperitoneal injection of 4% thioglycollate solution (Difco). Peritoneal cells were plated, nonadherent cells were removed, and medium was replaced with M199 (without phenol red), including the plasmin substrate S-2390 (Chromogenix) and human Glu-plasminogen (American Diagnostica). Plasminogen activation was measured as the change in absorbance at 405 nm and the level of uPA expression calculated as \( \Delta \text{OD}_{405} / \text{h}^2 \).\textsuperscript{15,21}

**Aortic uPA Activity**

After 5 or 10 weeks on the atherogenic diet, anesthetized mice were perfused with saline, and their thoracic aortas were snap-frozen and stored at −80°C. Aortas were pulverized and homogenized in 400 \( \mu \text{L} \) of buffer consisting of (in mmol/L) 75 acetic acid, pH 4.2, 75 KCl, 225 NaCl, 10 EDTA, and 100 arginine, and 0.25% Triton X-100, and centrifuged at 12,000g. The supernatant was frozen at −80°C. Protein concentration in the extracts was determined with the BCA assay (Pierce), and uPA activity was measured in aliquots diluted to 0.8 \( \mu \text{g/mL} \) protein. To convert single-chain uPA in the extracts to active 2-chain uPA, 20 \( \mu \text{L} \) of extract was added to 100 \( \mu \text{L} \) of buffer including 50 mmol/L Tris, pH 8.8, 38 mmol/L NaCl, 0.1% BSA, and 10 \( \mu \text{g/mL} \) human plasmin (American Diagnostica). After 30 minutes at 37°C, 2 \( \mu \text{L} \) of aprotinin (1.2 trypsin inhibitor units/mL, Sigma) was added to inhibit plasmin and other proteases. The uPA substrate S-2444 (Chromogenix) was added at 0.48 mg/mL. Absorbance at 405 nm was measured immediately and after 17 hours. Conversion of single-chain uPA to 2-chain uPA by plasmin treatment and addition of aprotinin maximizes specificity for uPA.\textsuperscript{15,22}

**Tissue Processing and Histology**

Pinned aortas were stained with Sudan IV.\textsuperscript{23} For frozen sectioning, hearts and aortic roots were processed into OCT compound (VWR).\textsuperscript{24} Other hearts were placed overnight in fixative, then processed into paraffin.

Eighty 10-\( \mu \text{m} \) thick serial cryosections were cut from each aortic root, beginning at the level of attachment of the aortic valve cusps. Serial sections were stained with hematoxylin and eosin, oil red O, Movat’s pentachrome, and the macrophage-specific antibody MOMA-2 (Biosource). Sections of paraffin-embedded hearts were stained with hematoxylin and eosin or Masson’s trichrome.

Aortic root sections from 4 transgenic and 5 nontransgenic mice (all \textit{Apoe} \textsuperscript{-/-}) were stained with an antibody to smooth muscle \( \alpha \)-actin (Clone 1A4, Dako) or control mouse IgG directed against an irrelevant epitope (Dako U 0951).

**Quantification of Coronary Artery Stenosis**

Two observers, blinded to genotype, evaluated all oil red O-stained and MOMA-2-stained areas, and lumen circumference were measured on 4 to 8 evenly spaced step sections per aortic root by use of computer-assisted color thresholding and planimetry.\textsuperscript{24} Total luminal surface and stained areas of Sudan IV–treated, pinned aortas were quantified.\textsuperscript{25}

**Peripheral Blood Cell Counts**

Counts were performed by an outside laboratory (Phoenix Central Laboratory).

**Statistical Methods**

Group means were compared by unpaired \( t \) test, except for percentage stenosis, for which the rank-sum test was used. Accuracy of genotyping based on assessment of medial destruction was evaluated by \( \chi^2 \) testing. Survival probabilities were compared by the log-rank test.\textsuperscript{26,27}

**Results**

**Expression of uPA in Founder Lines**

Of 28 pups, 5 transmitted the transgene to their offspring. To determine the level and extent of macrophage-specific uPA expression, freshly harvested organs and thioglycollate-stimulated, cultured peritoneal macrophages were studied by Northern analysis. Macrophages were also assayed for plas-
phages. SR-uPA transgene is expressed predominantly in macrophages. We identified 3 lines with varying levels of macrophage-specific SR-uPA transgene expression (Figure 1, and data not shown). Later experiments, performed after the 3 lines had been crossed into the Apoe^{-/-} background, showed that only the line with the highest macrophage-specific uPA expression (Figure 1) also had elevated uPA activity in the atherosclerotic aorta. In addition, only this line showed an effect of uPA expression on atherosclerosis (Table, and data not shown). Here, we report data generated with this line of mice.

uPA activity (Figure 1) and SR-uPA mRNA expression in thioglycollate-stimulated, ex vivo macrophages of this line of mice were substantial. uPA mRNA was increased in thioglycollate-stimulated peritoneal macrophages of a nontransgenic mouse compared. Moreover, the ex vivo assay system itself is nonphysiological (stimulated macrophages grown on plasmin substrate). In addition, there is no well-established quantitative relationship between peritoneal macrophage gene expression ex vivo and aortic wall activity of transgenic and nontransgenic macrophages suggested that uPA activity in the transgenic peritoneal macrophages was elevated by as much as 300-fold. We were concerned that this level of macrophage uPA overexpression might be nonphysiological. However, there are no data on uPA expression in stimulated ex vivo human peritoneal macrophages with which these results could be compared. Moreover, the ex vivo assay system itself is nonphysiological (stimulated macrophages grown on plastic in serum-free medium). In addition, there is no well-established quantitative relationship between peritoneal macrophage gene expression ex vivo and aortic wall macrophages (200±60% versus 5±2% of GAPDH mRNA signal, n=4). Use of ΔOD_{405}/h as OD_{405} of medium. Data are mean±SD.

<table>
<thead>
<tr>
<th>Comparison of Transgenic (SR-uPA^{+/+}) and Nontransgenic (SR-uPA^{0/0}) Mice</th>
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<tr>
<td>Peripheral blood monocytes, per µL</td>
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<td>Peripheral blood monocytes, % total leukocytes</td>
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<td>Aortic uPA activity, IU/mg</td>
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<td>5-wk diet</td>
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<td>Plasma cholesterol, mg/dL</td>
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<td>Aortic root intimal area, µm²×10^6</td>
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<td>9.5±3.2‡ (n=16)</td>
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<td>Aortic root oil red 0 area, µm²×10^4</td>
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<td>6.2±2.1‡ (n=16)</td>
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<td>Aortic root oil red 0 area, % of intimal area</td>
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<td>Aortic root MOMA-2 area, µm²×10^5</td>
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<td>4.2±2.4‡ (n=13)</td>
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<td>Aortic root MOMA-2 area, % of intimal area</td>
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<tr>
<td>Total aortic lumen surface area, mm²</td>
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<td>64±5.8 (n=10)</td>
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<td>Sudanophilic lesions, % of total aortic lumen surface area</td>
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<td>Aortic root lumen circumference, mm</td>
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<td>Maximal coronary stenosis, % of lumen narrowing</td>
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Values are mean±SD except for coronary stenoses, which are median and (25%–75%) range. n indicates mice in each group except for coronary stenosis, for which n indicates arteries. Variations in n between groups are because of omission of specimens of suboptimal technical quality.

P values are for difference from nontransgenic controls: *P≤0.05; †P≤0.01; ‡P≤0.001.
macrophage gene expression in vivo. For these reasons, we bred the transgene into the C57BL/6 Apoe<sup>−/−</sup> background and measured uPA activity in extracts of atherosclerotic aortas. Because uPA expression and activity in atherosclerotic human arteries is elevated 2- to 5-fold, there is a reference range with which aortic uPA activity in the transgenic mice could be compared.

**uPA Activity Is Significantly Elevated in Aortas of SR-uPA Apoe<sup>−/−</sup> Mice**

uPA activity was significantly elevated in aortas of transgenic SR-uPA Apoe<sup>−/−</sup> mice (Table). The magnitude of elevated uPA activity in 10-week transgenic aortas (2.2-fold) was within the range reported in atherosclerotic human arteries. There is a reference range with which aortic uPA activity in the transgenic mice could be compared.

**SR-uPA Apoe<sup>−/−</sup> Mice Have Unaltered Plasma Lipid Profiles**

SR-uPA Apoe<sup>−/−</sup> mice had no significant alterations in plasma cholesterol, triglycerides, or FPLC profiles (Table; online Data Supplement).

**Peripheral Blood Monocyte Counts**

Neither total peripheral monocyte counts nor percentage of peripheral blood monocytes differed between transgenic and nontransgenic mice (Table; P≥0.8 for both comparisons).

**Increased Atherosclerosis in SR-uPA Apoe<sup>−/−</sup> Mice**

After 10 weeks on diet, both intimal area in aortic root sections and sudanophilic area on pinned aortas were significantly increased in transgenic mice (2-fold; Table; Figure 2, A and B).

### Plaque Structure and Composition Are Unaltered in SR-uPA Apoe<sup>−/−</sup> Mice

Total plaque macrophage and lipid areas in aortic root cross sections were both significantly increased in transgenic mice (2-fold; Table). However, transgenic plaques were also proportionately larger; therefore, the percentage of plaque occupied by macrophages and lipids did not differ between transgenic and nontransgenic mice (Table; P=0.3 for both). Smooth muscle α-actin staining was essentially limited to a thin layer along the surface of some of the plaques and did not differ between transgenic and nontransgenic mice. No differences in collagen, proteoglycan, or elastin staining were discernible in Movat-stained sections of transgenic and nontransgenic intimal plaques.

**SR-uPA Apoe<sup>−/−</sup> Mice Have Dilated Aortic Roots and Increased Medial Destruction**

Aortic root lumen circumferences were significantly larger in transgenic mice. This dilation was only focal, however, because the total luminal surface area of transgenic aortas was not increased (Figure 2, A and B, and the Table). The appearance of the media in individual aortic root sections ranged from essentially intact to severe focal destruction (Figure 2, C and D). Medial destruction (loss of both matrix and smooth muscle cells) appeared more severe in transgenic mice. To evaluate this impression objectively, an observer blinded to genotype was given a single Movat-stained aortic root section from each of 7 transgenic and 7 nontransgenic mice and asked to assign a genotype based only on the extent of medial destruction. Twelve of 14 mice (86%) were correctly genotyped (P<0.01).

**SR-uPA Apoe<sup>−/−</sup> Mice Have Severe Occlusive Proximal Coronary Artery Disease, Extensive Myocardial Infarcts, and Early Mortality**

We enrolled mice in a longer-term study to determine whether they developed aneurysms or plaque rupture. However, transgenic mice began to die suddenly beginning at ~11 weeks of age (Figure 3). Mice that died suddenly or were killed at 15 weeks of age (for the atherosclerosis study) had gross and histological evidence of myocardial infarcts (Figure 4, A and C) as well as significant stenoses and total occlusions of proximal coronary arteries (Figure 4, D through
null mice might instead result from secondary, systemic complications of atherosclerosis. Alternatively, accelerated atherosclerosis in plasminogen-deficient mice (which have increased arterial wall plasminogen and PAI-1 and with mice overexpressing collagenase in macrophages) suggests that atherosclerosis may be accelerated either by an excess of plasmin activity or by an increase in uPA-mediated proteolytic activity in vivo in aortic wall macrophages. Our major findings were as follows: (1) Transgenic mice expressing elevated levels of uPA in ex vivo macrophages and in the atherosclerotic artery wall; (2) uPA overexpression in the aorta caused a 2-fold increase in atherosclerosis but did not alter plaque structure; (3) uPA-overexpressing mice had increased medial destruction and aortic root dilation; and (4) uPA-overexpressing mice died suddenly and prematurely, with severe proximal coronary atherosclerosis and myocardial infarcts. Therefore, macrophage-expressed uPA contributes to the progression and complications of atherosclerosis.

Our results must be reconciled with atherosclerosis studies performed with mice deficient in uPA, plasminogen, or PAI-1 and with mice overexpressing collagenase in macrophages. First, mice deficient in uPA had unaltered atherosclerosis. In contrast, uPA overexpression in macrophages increases atherosclerosis. uPA may accelerate atherosclerosis only when it is expressed above a critical level. Other factors that may explain why uPA deficiency did not decrease atherosclerosis include use of a more atherogenic diet (1.25% cholesterol with added cholic acid) and the apparent use of mice with mixed genetic backgrounds. Second, Apoe<sup>−/−</sup> mice deficient in plasminogen had increased atherosclerosis. In contrast, the SR-uPA mice (which have increased arterial wall plasminogen activation) have increased atherosclerosis. This result suggests that atherosclerosis may be accelerated either by an absence of plasmin or by an excess of plasmin activity. Alternatively, accelerated atherosclerosis in plasminogen-null mice might instead result from secondary, systemic effects of the wasting disease that these mice develop. Third, our results are consistent with a recent study showing increased atherosclerosis in PAI-1 null mice. Finally, mice overexpressing collagenase in macrophages had elevated artery wall proteolytic activity but less atherosclerosis. In contrast, elevated artery wall proteolytic activity in SR-uPA mice increased lesion size. This discrepancy is informative because it confirms the specificity of our results. Accelerated atherosclerosis in SR-uPA mice is not a nonspecific effect of protease overexpression in macrophages; rather, it is a specific effect of uPA overexpression.

The inconsistency between the substantial peritoneal macrophage uPA overexpression detected in SR-uPA and the modest uPA overexpression measured in aortic extracts (Table) is striking. Another line of SR-uPA transgenic mice also showed a large discrepancy between peritoneal macrophage uPA expression (estimated 10-fold increase) and uPA expression in aortic tissue (no increase). Although it is probable that the absolute level of uPA activity (IU/mg protein) is higher within the macrophage-rich arterial lesions than in whole aortic extracts, it is also likely that the ex vivo assay, performed in nonphysiological conditions using peritoneal macrophages, overestimates uPA expression in vivo in aortic wall macrophages. Alternatively, and far less likely, a tremendous increase in uPA-mediated proteolytic activity in vivo increases atherosclerosis only modestly and is insufficient to alter plaque structure.

There are several mechanisms through which uPA could accelerate atherosclerosis. uPA might increase macrophage migration into the intima. uPA could increase cell proliferation by activating or releasing matrix-bound growth factors. uPA might also bind to receptors on vascular cells, stimulating proliferation and migration. uPA-stimulated proteolysis could increase lipid accumulation by cleaving artery wall matrix proteins, creating binding sites for atherogenic lipoproteins. It is likely that uPA is acting locally, within the plaque, to accelerate atherosclerosis, because among the 3 lines of mice that we studied, only the line with elevated artery wall uPA expression had increased atherosclerosis. Systemic effects of uPA are less likely, because there was no effect of uPA
overexpression on plasma lipids (Table; online Data Supple-
mment), and we did not encounter neonatal bleeding or hepato-
cellular carcinoma, which occur in mice with general-
ized uPA overexpression.34,35

To identify a cause for the sudden deaths of the transgenic Apoe−/− mice, we examined several organs postmortem. Only the hearts and aortas appeared abnormal, and we did not observe either peripheral edema or aortic rupture. Thus, the deaths were not caused by chronic heart failure or aneurysmal disease. The presence of myocardial infarcts along with occlusive coronary disease suggests that the deaths were caused by cardiac arrhythmias. Further studies may reveal whether the SR-uPA Apoe−/− mice are a useful model of sudden death caused by atherosclerotic cardiovascular disease.

The combination of elevated macrophage proteolytic ac-
tivity, sudden death, and coronary occlusions suggested that macrophage-expressed uPA might cause plaque rupture. However, the occlusions appeared to result from aortic plaque extension into coronary ostia (Figure 4, D through F). Plaque rupture may not have occurred because the lesions were at an early stage, without necrotic cores. Mice with macrophage-
specific expression of collagenase also did not manifest plaque rupture.13 It remains a challenge to provide experi-
mental support for the hypothesis that elevated lesion proteo-
lytic activity causes plaque rupture.

The finding of dilated aortic roots in SR-uPA mice sup-
ports a role for artery wall uPA activity in aneurysm forma-
tion. The absence of frank aortic aneurysms is most likely a result of the early deaths of SR-uPA mice. The aortic root dilation contrasts with a previous study in which brief overexpression of uPA in endothelial cells of rabbit carotid arteries caused arterial constriction.15 There are 2 likely reasons for this. First, the vascular media in the present study may have been sufficiently damaged by uPA overexpression (Figure 2D) that it could no longer sustain contractile activity. Alternatively, constriction could be caused by an endothelial cell–specific activity of uPA, such as cleavage of a vasodi-
lator peptide.36

In summary, our data support the hypothesis that elevated uPA expression in arterial wall macrophages contributes to occlusive vascular disease and to aneurysm formation. SR-

uPA mice may provide a useful model of sudden death associated with coronary atherosclerosis. Elevated arterial wall uPA activity in humans may be a target for therapies that slow atherosclerosis.

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References
1. Padró T, Emeis JJ, Steins M, et al. Quantification of plasminogen activ-
ators and their inhibitors in the aortic vessel wall in relation to the

presence and severity of atherosclerotic disease. Arterioscler Thromb
15:1444–1455.
3. Kienast J, Padro T, Steins M, et al. Relation of urokinase-type plasmin-
ogen activator expression to presence and severity of atherosclerotic
fibroinolytic activators and inhibitors with macrophages in atherosclerotic
genenes in atherosclerotic abdominal aortic aneurysm wall: a possible
collagenolysis by interstitial collagensases-1 and -3 in vulnerable human
vessel wall disease in mice predisposed to atherosclerosis. Proc Natl Acad
LDL receptor–deficient and apolipoprotein B-deficient mice is inde-
dependent of genetic alterations in plasminogen activator inhibitor-1. Arte-
deficiency protects against atherosclerosis progression in the mouse carotid
inhibitor-1 promotes growth and abnormal matrix remodeling of
advanced atherosclerotic plaques in apolipoprotein E–deficient mice.
expressing human matrix metalloproteinase-1 in macrophages have less
14. Bendeck MP. Matrix metalloproteinases: are they antiatherogenic but
15. Falkenberg M, Tom C, DeYoung MB, et al. Increased expression of
urokinase during atherosclerotic lesion development causes arterial con-
striction and lumen loss, and accelerates lesion growth. Proc Natl Acad
elements target gene expression to macrophages and to foam cells of
17. Degen SJF, Heckel JL, Reich E, et al. The murine urokinase-type plas-
central nervous system: characterization of the lipoproteins in cerebro-
spinal fluid and identification of apolipoprotein B-LDL (LDL) receptors in
in mice causes abnormal yolk sac vasculo-
20. Dichek DA, Lee SW, Nguyen NH. Characterization of recombinant
plasminogen activator production by primate endothelial cells transduced
21. Friberger P. Chromogenic peptide substrates: their use for the assay of
factors in the fibrinolytic and the plasma kallikrein–kinin systems. Scand J
22. Véniant MM, Pierotti V, Newland D, et al. Susceptibility to atheroscle-
rosis in mice expressing exclusively apolipoprotein B48 or apolipoprotein
23. Gosling J, Slaymaker S, Gu L, et al. MCP-1 deficiency reduces suscepti-
bility to atherosclerosis in mice that overexpress human apolipoprotein
hyperlipidemic mice deficient in α-tocopherol transfer protein and


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