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 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{15} to the mouse uPA gene\textsuperscript{27} (from J. Degen, Children’s Hospital Medical Center, Cincinnati, Ohio). This “SR-uPA” transgene was used to generate transgenic mice in the C57BL/6\times SJL 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 Apoe\textsuperscript{−/−} background by serial breedings with C57BL/6 Apoe\textsuperscript{−/−} mice (Jackson Laboratories, Bar Harbor, Maine). SR-uPA 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 (≤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} The reliability of conversion of single-chain to 2-chain uPA and the linearity and sensitivity of the uPA assay were confirmed by assay of human single-chain uPA (American Diagnostica) in parallel.

**Tissue Processing and Histology**

Pinned aortas were stained with Sudan IV.\textsuperscript{21} 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-μm-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 Apoe\textsuperscript{−/−}) were stained with an antibody to smooth muscle α-actin (Clone 1A4, Dako) or control mouse IgG directed against an irrelevant epitope (Dako U 0951).

**Morphometry**

Total intimal area, intimal 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 quantitated.\textsuperscript{58}

**Quantification of Coronary Artery Stenosis**

Two observers, blinded to genotype, evaluated all oil red O–stained aortic root sections to identify the major epicardial coronary arteries and locate, for each artery, the section with the maximal stenosis. Images of these sections were used to measure, along a diameter extending across the maximal stenosis, the original lumen diameter (ie, distance between the inner edges of the media) and the residual lumen diameter. Percent stenosis=(original lumen diameter−residual lumen diameter/original lumen diameter)×100%.

**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 measurement of medial destruction was evaluated by χ² 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. Data are representative of 2 independent experiments, performed with RNA from different mice (4 total). A third experiment, with 8 additional mice, further quantified uPA mRNA levels in macrophages (see Results). B, Plasminogen activator activity. Thioglycollate-stimulated peritoneal macrophages of transgenic (SR-uPA; n=4) and nontransgenic littermate control mice (n=2; essentially identical results were obtained with 5 other nontransgenic mice). Ex vivo macrophages were incubated with plasminogen and plasmin substrate S-2390, and plasmin activity was measured as OD405 of H11005 and nontransgenic control mice (n=2; essentially identical results were obtained with 5 other nontransgenic mice). Ex vivo macrophages were incubated with plasminogen and plasmin substrate S-2390, and plasmin activity was measured as OD405 of H11005 and nontransgenic control mice (n=2; essentially identical results were obtained with 5 other nontransgenic mice). Ex vivo macrophages were incubated with plasminogen and plasmin substrate S-2390, and plasmin activity was measured as OD405 of medium. Data are mean±SD.

Comparison of Transgenic (SR-uPA+/+) and Nontransgenic (SR-uPA−/−) Mice

<table>
<thead>
<tr>
<th></th>
<th>SR-uPA+/+</th>
<th>SR-uPA−/−</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peripheral blood monocytes, per µL</td>
<td>160±117 (n=5)</td>
<td>195±259 (n=5)</td>
</tr>
<tr>
<td>Peripheral blood monocytes, % total leukocytes</td>
<td>1.8±1.5 (n=5)</td>
<td>1.8±2.2 (n=5)</td>
</tr>
<tr>
<td>Aortic uPA activity, IU/mg</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5-wk diet</td>
<td>0.20±0.047* (n=5)</td>
<td>0.136±0.038 (n=6)</td>
</tr>
<tr>
<td>10-wk diet</td>
<td>0.35±0.087‡ (n=8)</td>
<td>0.16±0.037 (n=8)</td>
</tr>
<tr>
<td>Plasma cholesterol, mg/dL</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5-wk diet</td>
<td>1193±344 (n=6)</td>
<td>1496±104 (n=6)</td>
</tr>
<tr>
<td>10-wk diet</td>
<td>1073±279 (n=8)</td>
<td>1309±179 (n=9)</td>
</tr>
<tr>
<td>Aortic root intimal area, µm²×10⁵</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5-wk diet</td>
<td>9.5±3.2‡ (n=16)</td>
<td>4.3±1.3 (n=11)</td>
</tr>
<tr>
<td>10-wk diet</td>
<td>6.2±2.1‡ (n=16)</td>
<td>2.5±0.51 (n=11)</td>
</tr>
<tr>
<td>Aortic root intimal area, % of intimal area</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5-wk diet</td>
<td>65±9.7 (n=16)</td>
<td>61±15 (n=11)</td>
</tr>
<tr>
<td>10-wk diet</td>
<td>4.2±2.4‡ (n=13)</td>
<td>1.6±0.69 (n=10)</td>
</tr>
<tr>
<td>Aortic root MOMA-2 area, µm²×10⁵</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5-wk diet</td>
<td>44±11 (n=13)</td>
<td>38±15 (n=10)</td>
</tr>
<tr>
<td>10-wk diet</td>
<td>64±5.8 (n=10)</td>
<td>61±4.4 (n=9)</td>
</tr>
<tr>
<td>Sudanophilic lesions, % of total aortic lumen surface area</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5-wk diet</td>
<td>6.0±3.3‡ (n=10)</td>
<td>3.3±1.1 (n=9)</td>
</tr>
<tr>
<td>Aortic root lumen circumference, mm</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5-wk diet</td>
<td>5.7±0.68* (n=16)</td>
<td>5.2±0.28 (n=11)</td>
</tr>
<tr>
<td>Maximal coronary stenosis, % of lumen narrowing</td>
<td>62‡ (40–83) (n=23)</td>
<td>2.6 (0–17) (n=17)</td>
</tr>
</tbody>
</table>

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.
Lipid Profiles transgenic mice could be compared. In aortic root tissues, the level of uPA expression and activity in atherosclerotic tissues is elevated 2- to 5-fold,1,3 there is a significant increase in uPA activity in 10-week transgenic aortas (2.2-fold) was within the range reported in atherosclerotic human arteries.1,3 In aortic root tissues, the level of uPA expression and activity in atherosclerotic tissues is elevated 2- to 5-fold,1,3 there is a significant increase in uPA activity in 10-week transgenic aortas (2.2-fold) was within the range reported in atherosclerotic human arteries.1,3

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

The uPA activity in aortas of transgenic mice was significantly elevated 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. No differences in collagen, proteoglycan, or elastin staining were discernible in Movat-stained sections of transgenic and nontransgenic intimal plaques.

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

SR-uPA Apoe−/− 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−/− 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−/− 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. No differences in collagen, proteoglycan, or elastin staining were discernible in Movat-stained sections of transgenic and nontransgenic intimal plaques.

**SR-uPA Apoe−/− 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−/− 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. Third, our results are consistent with a recent study of uPA-stimulated proteolysis could increase lipid accumulation (1.25% cholesterol with added cholic acid) and the apparent use of mice with mixed genetic backgrounds. Second, Apoe−/− mice deficient in plasminogen had increased atherosclerosis. In contrast, elevated artery wall proteolytic activity but less atherosclerosis, because among the 3 lines of mice that we studied, only the line with elevated artery wall uPA expression (estimated 100-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 aortic lesions than in whole aortic extracts, it is also likely that the ex vivo assay, performed in nonphysiological conditions using peritoneal macrophages, overestimates uPA overexpression 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.

The inconsistency between the substantial peritoneal macrophage uPA overexpression detected ex vivo (Figure 1) 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 100-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 aortic lesions than in whole aortic extracts, it is also likely that the ex vivo assay, performed in nonphysiological conditions using peritoneal macrophages, overestimates uPA overexpression 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.

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.

Discussion
We expressed a uPA transgene in macrophages of Apoe−/− mice. Our major findings were as follows: (1) Transgenic mice expressed 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 and the apparent use of mice with mixed genetic backgrounds. Second, Apoe−/− 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.

F). The arterial lumens were occluded with foam cells and lipids; neither intraplaque hemorrhages nor intraluminal thrombi were present. To quantify proximal coronary atherosclerosis, we measured the maximal stenosis in each major coronary artery. Coronary stenoses were significantly more severe in transgenic mice (Table).

Figure 4. Cardiovascular pathology in SR-uPA Apoe−/− mice. A, Hearts of nontransgenic (non-tg) and SR-uPA mice. A large area of fibrosis is evident in right ventricular free wall of SR-uPA heart (arrows). The right atrial appendage (*) appears enlarged. B and C, Sections from hearts of nontransgenic and SR-uPA mice (Masson’s trichrome). Myocyte loss and fibrosis are present in SR-uPA heart. A transmural infarct extends from epicardial surface (arrowheads) to right ventricle (asterisk). Some surviving cardiac myocytes are indicated (arrows). D, Aortic root lesion in a SR-uPA mouse extending from sinus of Valsalva (Ao) into a coronary artery (Co). An ostial stenosis of ~50% is present (oil red O stain). E and F, Proximal coronary occlusions (arrows) in SR-uPA mice that died suddenly (hematoxylin and eosin). Lu indicates aortic lumen. Bars: A, 2 mm; B-F, 200 μm.
overexpression on plasma lipids (Table; online Data Sup-
plement), and we did not encounter neonatal bleeding or hepato
cellular carcinoma, which occur in mice with gen-
eralized uPA overexpression.34,35

To identify a cause for the sudden deaths of the transgenic 
Apo e−/− 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 
oclusive coronary disease suggests that the deaths were 
caused by cardiac arrhythmias. Further studies may reveal 
whether the SR-uPA Apo e−/− 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. How-
ever, 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 macro-
phage-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 vaso-
dilator 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.

Acknowledgments

This work was supported by grants from the University of California 
Tobacco-Related Disease Research Program (7RT-0016), the Na-
tional Institutes of Health (NIH) (HL-61860 and HL-69063), and the 
Howard Hughes Medical Institute Research Resources Program (to 
Dr D. Dichek). Dr DeYoung and Dr H. Dichek were supported by 
awards from the NIH (F32-HL-10022 and K08-HL-04031). The 
authors thank Brian Kelley and Ruth Linnemann for assistance with 
experiments and Gary Howard, Stephen Ordway, and Margo Weiss 
for editorial advice and assistance.

References

1. Padró T, Emeis JJ, Steins M, et al. Quantification of plasminogen acti-
vators 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 

fibrolytic activators and inhibitors with macrophages in atherosclerotic 

genesis in atherosclerotic abdominal aortic aneurysm wall: a possible 

6. Lijnen HR. Plasmin: matrix metalloproteinases in vascular 

collagenolysis by interstitial collagenases-1 and -3 in vulnerable human 

activates matrix metalloproteinases during aneurysm formation. Nat 

vessel wall disease in mice predisposed to atherosclerosis. Proc Natl Acad 

LDL receptor–deficient and apoprotein E–deficient mice is inde-
pendent 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. 

xpressing 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(E) (LDL) receptors in 

knockout 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 

22. Véniant MM, Pierotti V, Newland D, et al. Susceptibility to atheroscle-
rosis in mice expressing exclusively apolipoprotein B48 or apolipoprotein 

23. Véniant MM, Pierotti V, Newland D, et al. Susceptibility to atheroscle-
rosis in mice expressing exclusively apolipoprotein B48 or apolipoprotein 

24. Gosling J, Slaymaker S, Gu L, et al. MCP-1 deficiency reduces suscep-
tibility to atherosclerosis in mice that overexpress human apolipoprotein 

hyperlipidemic mice deficient in α-tocopherol transfer protein and 
Macrophage-Targeted Overexpression of Urokinase Causes Accelerated Atherosclerosis, Coronary Artery Occlusions, and Premature Death
Aaron E. Cozen, Hideaki Moriwaki, Michal Kremen, Mary Beth DeYoung, Helén L. Dichek, Katherine I. Slezicki, Stephen G. Young, Murielle Véniant and David A. Dichek

_Circulation_. 2004;109:2129-2135; originally published online April 19, 2004; doi: 10.1161/01.CIR.0000127369.24127.03
_Circulation_ is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2004 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7322. Online ISSN: 1524-4539

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circ.ahajournals.org/content/109/17/2129

Data Supplement (unedited) at:
http://circ.ahajournals.org/content/suppl/2004/05/03/01.CIR.0000127369.24127.03.DC1

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in _Circulation_ can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to _Circulation_ is online at:
http://circ.ahajournals.org//subscriptions/