Cystatin C Deficiency Increases Elastic Lamina Degradation and Aortic Dilatation in Apolipoprotein E–Null Mice

Galina K. Sukhova, Bing Wang, Peter Libby, Jie-Hong Pan, Yaou Zhang, Anders Grubb, Kenneth Fang, Harold A. Chapman, Guo-Ping Shi

Abstract—The pathogenesis of atherosclerosis and abdominal aortic aneurysm involves substantial proteolysis of the arterial extracellular matrix. The lysosomal cysteine proteases can exert potent elastolytic and collagenolytic activity. Human atherosclerotic plaques have increased cysteine protease content and decreased levels of the endogenous inhibitor cystatin C, suggesting an imbalance that would favor matrix degradation in the arterial wall. This study tested directly the hypothesis that impaired expression of cystatin C alters arterial structure. Cystatin C–deficient mice (Cyst C−/−) were crossbred with apolipoprotein E–deficient mice (ApoE−/−) to generate cystatin C and apolipoprotein E–double deficient mice (Cyst C−/−/ApoE−/−). After 12 weeks on an atherogenic diet, cystatin C deficiency yielded significantly increased tunica media elastic lamina fragmentation, decreased medial size, and increased smooth muscle cell and collagen content in aortic lesions of ApoE−/− mice. Cyst C−/−/ApoE−/− mice also showed dilated thoracic and abdominal aortae compared with control ApoE−/− mice, although atheroma lesion size, intimal macrophage accumulation, and lipid core size did not differ between these mice. These findings demonstrate directly the importance of cysteine protease/protease inhibitor balance in dysregulated arterial integrity and remodeling during experimental atherogenesis. (Circ Res. 2005;96:368-375.)

Key Words: cystatin C ● apolipoprotein E ● cysteine protease ● atherosclerosis ● smooth muscle cells

Atherogenesis involves substantial remodeling of the arterial extracellular matrix.1 Considerable evidence links degradation of elastic laminae and of interstitial collagen with migration of leukocytes and smooth muscle cells (SMCs), neointima formation, atherosclerotic plaque rupture, and to arterial ectasia and aneurysm formation.

We previously proposed a role for cysteine proteases, in particular cathepsins S and K, in atherosclerosis. Human atheromatous plaque exhibits substantially increased expression of cathepsins S and K compared with normal vessels, and cathepsin S–positive SMCs colocalize with sites of elastic lamina fragmentation in the tunica media.2 Human vascular SMCs and endothelial cells express cathepsin S–dependent elastolytic and collagenolytic activity in response to proinflammatory cytokines or growth factors in vitro.2,3 Our recent finding of attenuated atherogenesis in cathepsin S–deficient mice provided direct evidence for cysteine protease involvement in atherogenesis.4 Mice deficient in cathepsin S had 50% less atherosclerosis than controls at both 8 and 12 weeks of atherogenic diet.

Importantly, we found a reciprocal relationship between the levels of cathepsins tested and their most abundant endogenous inhibitor cystatin C in diseased human arterial tissue. Both atherosclerotic and aneurysmal human lesions show reduction of cystatin C compared with normal arteries,5 indicating an imbalance in cysteine proteases and their inhibitor in human arterial disease.2 Eriksson et al6 recently demonstrated that a human cystatin C promoter polymorphism mutation, associated with reduced plasma cystatin C, correlated with a higher than average number of angiographically evident stenosis per coronary artery segment in survivors of myocardial infarction. Furthermore, Lindholt et al7 and our group5 independently showed a significant reduction of cystatin C level in the serum of patients with dilated abdominal aortas, highlighting the potential function of cystatin C not only in the local vascular microenvironment but systemically as well. Together, these findings suggest that cystatin C expression and its balance with its cognate proteases critically regulate arterial remodeling in atherosclerosis and aortic aneurysm progression. This study introduced inactivated cystatin C mutant alleles into apolipoprotein E–deficient (ApoE−/−) mice, which develop plaques that recapitulate many features of human atherogenesis,8 to test directly the importance of cysteine protease/cystatin C imbalance in atherogenesis and aortic ectasia.

Original received October 5, 2004; revision received January 4, 2005; accepted January 4, 2005.
From the Donald W. Reynolds Cardiovascular Clinical Research Center, Department of Medicine, Brigham and Women’s Hospital/Harvard Medical School, Boston, Mass; the Department of Medicine (G.K.S., B.W., P.L., G.-P.S.), University of California San Francisco (J.-H.P., Y.Z., K.F., H.A.C.), Calif; and the Department of Clinical Chemistry (A.G.), University of Lund, Sweden.
Correspondence to Guo-Ping Shi, DSc, Cardiovascular Medicine, NRB-7, 77 Ave Louis Pasteur, Boston, MA 02115. E-mail gshi@rics.bwh.harvard.edu
© 2005 American Heart Association, Inc.
Circulation Research is available at http://www.circresaha.org DOI: 10.1161/01.RES.0000155964.34150.F7

368
Materials and Methods

Cyst C<sup>−/−</sup>ApoE<sup>−/−</sup> Mice Generation and Animal Protocol

ApoE<sup>−/−</sup> (C57/B16) and Cyst C<sup>−/−</sup> mice (C57/B16/S129; Jackson Laboratories, Bar Harbor, Me)<sup>5</sup> were bred to generate Cyst C<sup>−/−</sup>ApoE<sup>−/−</sup> mice, which were interbred to generate ApoE<sup>−/−</sup>, Cyst C<sup>−/−</sup>ApoE<sup>−/−</sup>, and Cyst C<sup>−/−</sup> littersmates to avoid confounding caused by differences in genetic background. Six-week-old Cyst C<sup>−/−</sup>ApoE<sup>−/−</sup>, ApoE<sup>−/−</sup>, and Cyst C<sup>−/−</sup> male mice consumed an atherogenic diet.<sup>10</sup> After 8 and 12 weeks of a “Western” type cholate-free diet, mouse plasma was collected for plasma lipoprotein measurement and mouse aortae were harvested for lesion characterization, as described previously.<sup>4</sup>

Mouse Aorta Excision, Processing, and Evaluation

The aortic arch and abdominal aorta were prepared according to our standard protocol, as described previously.<sup>12–14</sup> Briefly, after opening the body, all mice were perfused at physiological pressure with normal saline via the left ventricle, and the adventitia was removed carefully before excising the heart-aorta complex (from the iliac bifurcation to the attached heart). The aortic arch was embedded in OCT for frozen section preparation.

Evaluation of atherosclerosis in mice used longitudinal sections of aortic arches as well as en face preparations of thoracic-abdominal aortae. Lesion size (intima and media), percentage of total area stained for SMCs, macrophages, interstitial collagen, and lipid of the aortic arch were analyzed as described. A segment of the lesser curvature of the aortic arch (3-mm) was defined by a perpendicular dropped from a fiducial point (the right side of the innominate artery) and the adventitia was removed carefully before excising the heart-aorta complex (from the iliac bifurcation to the attached heart). The aortic arch was embedded in OCT for frozen section preparation.

To measure lesion sizes (intima and media), serial longitudinal sections of the aortic arch (30 μm) were analyzed microscopically and sections with maximal intimal thickness at the exact same location were used. Images captured by a digital imaging system were analyzed with Image-Pro Plus software (Media Cybernetics). The percentage of the total area with positive color was recorded for each section by two blinded observers. Data were presented as mean±SD, and differences between groups were determined with a nonparametric Mann-Whitney test. Probability values <0.05 were considered significant.

To examine elastase activity in aortic tissues, protein extract (60 μg) was incubated with [3H]-elastin (300 μg) for 48 hours. Soluble tritium released into the supernatant medium reflects elastolysis. Data are expressed as μg of degraded elastin/10<sup>6</sup> cells per 24 hours.

Immunohistochemistry

Serial longitudinal cryostat sections (6 μm) of mouse aortic arches were incubated with primary antibodies followed by biotinylated secondary antibodies and avidin-biotin complex (Vector, Burlingame, Calif.). The reaction was visualized with 3-amin-9-ethyl carbazole (DAKO). Slides were counterstained with Gill hematoxylin solution (Sigma).<sup>4</sup>

Antibodies included rabbit anti-mouse Cat S (1:90),<sup>11</sup> rabbit anti-human cystatin C (1:1000),<sup>4</sup> rat anti-mouse macrophage (Mac-3, 1:1000, Pharmingen, San Diego, Calif), and T-cell (CD4, 1:100, Pharmingen). For mouse SMC α-actin staining, primary antibody (FITC-conjugated α-actin, 1:500, Sigma, St Louis, Mo) was applied, followed by anti-FITC biotin-conjugated secondary antibody (1:400, Sigma).

Results

Cyst C<sup>−/−</sup>ApoE<sup>−/−</sup> Mice Generation and Atherogenesis

This study used ApoE<sup>−/−</sup> and Cyst C<sup>−/−</sup> mice to generate Cyst C<sup>−/−</sup>ApoE<sup>−/−</sup> breeding pairs for further interbreedings. The resulting Cyst C<sup>−/−</sup>ApoE<sup>−/−</sup>, ApoE<sup>−/−</sup>, and Cyst C<sup>−/−</sup> littersmates appeared normal in reproduction, body weight, and food/water consumption. To enhance atherogenesis, mice consumed the atherogenic diet for 8 or 12 weeks.<sup>16</sup> As in
human atheromata, atherosclerotic lesions of ApoE−/− mice had reduced expression of cystatin C (not shown). As expected, total serum cholesterol in ApoE−/− mice that consumed the atherogenic diet exceeded that of those fed normal chow (Table 1). Cyst C−/− ApoE−/− mice had modestly higher total cholesterol than did control mice when they consumed the atherogenic diet for 8 or 12 weeks. Cystatin C−/− deficient mice had lower levels of high-density lipoprotein (HDL) at the 8-week time point (Table 1), whereas plasma triglyceride levels remained similar at both time points tested.

### Increased Elastic Lamina Degradation in Cyst C−/−ApoE−/− Mice

We evaluated the degree of elastin degradation/fragmentation in longitudinal frozen sections from mouse aortae stained for elastica by Verhoeff-van Gieson (graded from 1 to 3, see Materials and Methods) (Figure 1A). Aortae from Cyst C−/−ApoE−/− mice showed significantly more elastic lamina fragmentation than those from ApoE−/− (2.7±0.8 versus 1.5±0.3; P<0.001; Figure 1B) or Cyst C−/− mice (2.7±0.8 versus 1.6±0.2; P<0.006).

Increased fragmentation of elastic laminae in cystatin C−/− atherosclerotic lesions could reflect enhanced levels of cysteinyld elastase activity. To test this hypothesis, we performed cysteine protease active site-labeling and elastase assay using protein extracts from freshly pulverized aortae of Cyst C−/−ApoE−/− and ApoE−/− control mice. Tissue extract [125I]-JPM labeling allowed us to determine the amounts of active cysteine proteases.15 Cyst C−/−ApoE−/− aortic extracts showed much higher levels of cathepsins S and

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### TABLE 1. Plasma Lipid Profile in Mice Fed With Chow or High Cholesterol Diet

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Diet</th>
<th>No.</th>
<th>Plasma Total Cholesterol, mg/dL</th>
<th>Plasma Total Triglyceride, mg/dL</th>
<th>Plasma Total HDL, mg/dL</th>
<th>Plasma Total LDL, mg/dL</th>
</tr>
</thead>
<tbody>
<tr>
<td>WT</td>
<td>Chow</td>
<td>10</td>
<td>112.9±15.4</td>
<td>162.1±18.0</td>
<td>52.4±12.3</td>
<td>28.1±7.8</td>
</tr>
<tr>
<td>Cyst C−/−</td>
<td>(12 weeks)</td>
<td>10</td>
<td>98.4±11.9</td>
<td>144.1±13.5</td>
<td>49.8±7.1</td>
<td>19.7±6.5</td>
</tr>
<tr>
<td>Cyst C−/−ApoE−/−</td>
<td>Chow</td>
<td>10</td>
<td>390.9±39.1</td>
<td>190.7±41.2</td>
<td>30.3±15.1</td>
<td>322.4±40.3</td>
</tr>
<tr>
<td>Cyst C−/−ApoE−/−</td>
<td>(12 weeks)</td>
<td>10</td>
<td>378.8±43.1</td>
<td>153.1±17.0</td>
<td>12.3±5.0</td>
<td>335.9±51.5</td>
</tr>
<tr>
<td>Cyst C−/−ApoE−/−</td>
<td>High cholesterol</td>
<td>15</td>
<td>472.3±44.4</td>
<td>146.1±33.0</td>
<td>32.7±12.6</td>
<td>410.3±40.0</td>
</tr>
<tr>
<td>Cyst C−/−ApoE−/−</td>
<td>(8 weeks)</td>
<td>16</td>
<td>578.5±93.6</td>
<td>125.5±22.2</td>
<td>23.8±6.7</td>
<td>529.7±97.3</td>
</tr>
<tr>
<td>Cyst C−/−ApoE−/−</td>
<td>High cholesterol</td>
<td>15</td>
<td>488.7±40.0</td>
<td>133.0±33.7</td>
<td>21.1±7.9</td>
<td>438.7±42.6</td>
</tr>
<tr>
<td>Cyst C−/−ApoE−/−</td>
<td>(12 weeks)</td>
<td>14</td>
<td>573.1±65.7</td>
<td>132.1±31.0</td>
<td>20.9±9.9</td>
<td>515.8±65.2</td>
</tr>
</tbody>
</table>

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**Figure 1.** Increased elastin degradation and cysteine protease expression and activity in aortic lesions of cystatin C−/−deficient mice. A, ApoE−/− mouse aortic arch Verhoeff van Gieson staining. Elastin degradation levels were graded from 1 to 3, as indicated in the left six panels with two magnifications. B, Aortic arches from Cyst C−/−ApoE−/− mice (n=10, open bar) showed significantly more elastin breakdown than control mice (n=11, filled bar). C, Active site-labeling illustrated increased expression of elastolytic cathepsins S, L, and B in Cyst C−/−ApoE−/− aortic lesions. Both long-chain (31 kDa) and short-chain (6 kDa) forms of cathepsin B are indicated.3 D, Significantly higher elastolytic activities were detected in aortic lesions from Cyst C−/−ApoE−/− mice (open bar) than control (filled bar).
L, two potent mammalian elastases, as well as the long-chain active form of cathepsin B (31 kDa) (Figure 1C). Consistent with this observation, extracts of aortae from Cyst C/ApoE mice had significantly higher ability to degrade insoluble elastin than extracts from aortae of ApoE wild type for Cyst C (Figure 1D).

Increased Intimal SMC and Collagen Content in Cyst C/ApoE Mice

Fragmentation of elastic laminae in Cyst C/ApoE mouse aortae might facilitate SMC migration from the tunica media and SMC accumulation in intimal lesions. To test this hypothesis, we stained sections of mouse aortae for α-actin (SMC content) and Picrosirius red (collagen). Lesions from Cyst C/ApoE mice had increased SMC content (% positive area) compared with ApoE mice (9.4±3.6 versus 4.2±2.0; P<0.002; Figure 2). Increased collagen content (% positive area) in intimal lesions of Cyst C/ApoE mice (14.4±4.9 versus 4.6±3.3; P<0.001; Figure 2) likely reflects accumulation of SMCs, the cell type responsible for the bulk of arterial extracellular matrix synthesis.

Increased SMC and collagen content in intimal lesions of Cyst C/ApoE mice may promote fibrous cap formation. We examined fibrous caps in all mice and graded them according to their content of SMCs and collagen (see Materials and Methods). By these criteria, Cyst C/ApoE mice had better developed fibrous caps than did ApoE mice (Figure 2, bottom right panel; 1.7±0.9 versus 0.9±0.5; P<0.05).

We also evaluated macrophage, T cell, and lipid content as well as the percentage of intimal area occupied by the lipid core, using longitudinal sections of aortic arches. Of these variables, lipid content decreased in Cyst C/ApoE intimal lesions compared with control (9.3±3% versus 15.2±6.6%; P=0.03), but the other features examined showed no significant change (macrophages, P=0.6; T cells, P<0.8; lipid cores, P<0.3).

Decreased Aortic Arch Tunica Media Thickness in Cyst C/ApoE Mice

Intima and media thickness were measured on 3-mm segments of longitudinal sections of aortic arches stained for SMCs (α-actin) and macrophages (Mac-3).14 We detected no significant changes in aortic arch intimal size or lesion severity (Table 2). However, mice deficient in cystatin C had...
thinning of the tunica media in the aortic arch: 0.23±0.05 mm² in Cyst C−/−ApoE−/− mice versus 0.3±0.05 mm² in ApoE−/− mice (Table 2; P<0.005).

**Expanded Abdominal Aortae in Cyst C−/−ApoE−/− Mice**

We found no significant difference in lipid deposition in abdominal aortae between Cyst C−/−ApoE−/− mice and their controls (% positive area, 9.2±4.8 versus 11±5.2; P=0.42), using an en face preparation of aorta explained previously.4,12,13 However, we detected significant expansion and elongation of the thoracic and abdominal aortae in Cyst C−/−ApoE−/− mice after 12 weeks of atherogenic diet. Both the aortic circumference at the level of the renal arteries (2.4±0.04 versus 2.1±0.05 mm; P<0.002) and the length of the aorta from the arch to the bifurcation (33±2.6 versus 30.5±1.7 mm; P<0.02) increased in ApoE−/− mice lacking cystatin C expression (Figure 3), but Cyst C−/−ApoE+/* mouse aortae (nonatherosclerotic) did not differ from ApoE−/− mice in either length (30.8±1.1 versus 30.5±1.7; P=0.55) or circumference (2.0±0.07 versus 2.1±0.05; P=0.67). The actual difference in aortic length and diameter between Cyst C−/−ApoE−/− and ApoE−/− or Cyst C−/−ApoE+/* mice may be underestimated because of postmortem aortic shrinkage, although all specimens were perfused at physiological pressure with saline for 2 to 3 minutes before excision, and were fixed immediately thereafter. Abdominal aortic expansion appeared quite consistent: 8 of 10 Cyst C−/−ApoE−/− mice had wider aortae (more than 2 mm), whereas only 1 of 11 ApoE−/− control mice reached this size. These observations may relate to the disrupted arterial elastin structure (Figure 1A and 1B), and the thinned tunica media detected in these mice (Table 2).

**Regulation of Cysteine Protease Expression by Aortic SMCs**

Both cathepsins S and K colocalized with SMCs at sites of internal elastic lamina fragmentation,2,4 regions of relatively deficient cystatin C expression.5 To test whether cystatin C regulates SMC cysteine protease activity, we performed [125I]-JPM active site-labeling using lysates of aortic SMCs isolated from Cyst C−/−ApoE−/− and control ApoE−/− mice. Quiescent SMCs showed no significant differences in expression of any known cysteine proteases, although cystatin C−/− mice expressed more of a higher molecular weight cysteine protease (∼45 kDa), possibly a cysteinyl cathepsin precursor (Figure 4A). However, Cyst C−/−ApoE−/− SMCs produced more active cathepsins B, L, and S than control cells after stimulation with proinflammatory cytokines or growth factors (Figure 4A). These findings suggest that cystatin C can influence cysteine protease expression and/or activity by vascular SMCs under inflammatory conditions.

**SMC Elastolytic/Collagenolytic Activity and Proliferation**

Cyst C deficiency also augmented the ability of aortic SMCs to degrade insoluble elastin in vitro. In fact, cultured Cyst C−/−ApoE−/− SMCs showed increased elastolytic and collagenolytic (against fluorescein-conjugated collagen-type I) activity. Cytokine or growth factor–treated or even unstimulated Cyst C−/−ApoE−/− SMCs exhibited enhanced levels of elastolytic/collagenolytic activity (Figure 4B and 4C). Although cell lysates from SMCs subjected to the same treatments also showed increased elastolytic/collagenolytic activity in Cyst C−/−ApoE−/− SMCs (not shown), the amount of extracellular matrix protein degraded was much lower, suggesting SMCs secrete the majority of the active elastolytic proteases.

At physiological concentrations, cystatin C inhibits T cell proliferation.4,13 Lack of cystatin C may thus alter vascular cell proliferation and further affect lesion cell content. Studies in mouse aortic SMCs tested this hypothesis. Cystatin C−/− deficient SMCs proliferated more rapidly than control cells (Figure 4D). If such increased SMC proliferation occurred in vivo it might contribute to the increased SMC and collagen content and fibrous cap development in Cyst C−/−ApoE−/− mouse atheromata (Figure 2).

**Figure 3.** Aortic expansion in Cyst C−/−ApoE−/− mice. En face preparations of mouse aortae from aortic arch to bifurcation were stained with oil-red O. Aortic circumference (adjacent to the renal arteries) and length (from aortic arch to bifurcation) were both enlarged in Cyst C−/−ApoE−/− mice (n=10, open bars) compared with ApoE−/− mice (n=11, filled bars).
Discussion
The ubiquitous protein cystatin C is the most efficient endogenous inhibitor of cathepsins S, K, and L, potent mammalian elastases, and collagenases.18 We previously detected significantly reduced expression of this protease inhibitor in human atherosclerotic and aneurysmal lesions compared with noninvolved normal arteries.9 Unlike the TIMPs, whose local levels change only modestly during atherogenesis,19–21 most SMC and macrophages in human atheromata lack cystatin C. However, both SMCs and macrophages in human atherosclerotic lesions express abundant cathepsins S and K,5 resulting in severe imbalance between cysteine proteases and their major inhibitor in these diseased arteries. As in the case of human atherosclerotic lesions, the atherosclerotic intima and the underlying tunica media in ApoE-deficient mice showed reduced cystatin C expression relative to normal aortae (not shown).

Although cystatin C–deficient mice show no overt abnormalities,9 mice lacking this protease inhibitor may have increased cysteine protease activity, especially animals with traumatic and/or other inflammatory conditions including atherogenesis. Some experiments in TIMP-deficient mice suggest such compensatory regulation. TIMP-3–deficient mice show increased MMP activity in the lung22 accompanied by spontaneous mouse airway enlargement.23 Deficiency of TIMP-1 led to significant loss of fibrillar collagens in mouse hearts, which amplified left ventricular remodeling during myocardial infarction24 and increased left ventricular end-diastolic volume and mass determined by echocardiography.25 These mice also had increased atheroma size, disrupted elastic laminae in the aortic tunica media, and developed more aortic medial ruptures on the ApoE−/− background after consumption of an atherogenic diet.24,26 The aortae of these TIMP-1−/−ApoE−/− animals showed particularly heightened MMP activity at sites of laminae elastin degradation.27

As anticipated, cystatin C deficiency in ApoE−/− mice yielded increased cysteiny1 elastase activity in aortae and disrupted arterial medial elastic laminae (Figure 1), similar to the sites of medial rupture in TIMP-1−/−ApoE−/− mice.26 but MMP activities were not affected (data not shown). Higher levels of cysteine protease activity in Cyst C−/−ApoE−/− mice could result from the absence of cystatin C as well as increased number of SMCs within the lesions, as these SMCs can express active elastolytic cysteine proteases (Figure 4A and 4B).2 This enhanced elastin fragmentation provides a potential mechanistic explanation for the increased SMC and collagen content of atherosclerotic lesions in these mice (Figure 2). Therefore, in normal arteries, abundant cystatin C, mostly produced by medial SMCs,5 participates in the homeostatic regulation of the integrity of the tunica media elastic laminae. The observed changes in aortic lesions of Cyst C−/−ApoE−/− mice, ie, increased cysteine protease activity (Figure 1C), enhancement of elastin degradation (Figure 1B and 1D), SMC accumulation in the intima (Figure 2), and thinning of the arterial tunica media (Table 2), thus appear to result from absence of cystatin C, rather than altered MMP activities. Supporting this hypothesis, gelatin zymography of aortic extracts from Cyst C−/−ApoE−/− mice showed no alteration of MMP activity relative to ApoE−/− control lesions (data not shown). The present experiments provide direct support for this hypothesis. Cystatin C–deficient SMCs expressed higher cathepsin L and S activity after stimulation by proinflammatory cytokines or growth factors (Figure 4A) typically found in human atheroma.28 Consistent with human SMCs,2 these cells exhibited enhanced degradation of extracellular elastin (Figure 4B) and collagen type-I (Figure 4C), particularly after exposure to proinflammatory stimuli. Such
increased cysteine protease activity and ECM degradation likely result from the lack of inhibitor.

Compared with cathepsin S–deficient mice,6 which showed reduced atherosclerotic lesion grades, decreased intimal lesion size, as well as lipid and macrophage content, we detected no changes in these variables in cystatin C–deficient mice. Instead, these mice demonstrated reduced size of the intima/medial tunica (Table 2) and enlargement of the aorta (Figure 3), findings not observed in hypercholesterolemic cathepsin S–deficient mice. Therefore, absence of cystatin C may affect activity of several cysteine proteases (eg, cathepsins B and L) whose functions in atherogenesis remain undetermined.

It remains unexplained why lack of cystatin C did not affect the atherosclerotic lesion grades or intimal sizes (Table 2), but did alter serum lipid profiles (Table 1). HDL levels seem more sensitive to cystatin C deficiency in ApoE−/− mice on chow diet or at early (8 weeks) stage of plaque formation on atherogenic diet. However, total cholesterol and LDL levels are higher in mice that consumed atherogenic diet at both time points. Recent data demonstrated that cysteine proteases might participate in lipid metabolism.29,30 Incubation of mouse peritoneal macrophages with recombinant cathepsin F or S led to rapid loss of preβ-HDL followed by cholesterol efflux reduction and lipid-free apoA-1 degradation in vitro.39 Further, cathepsins F, S, and K participate in LDL modification by degrading apoB-100, leading to enhanced LDL retention to human arterial proteoglycans.30 A similar function for cysteine proteases in metabolizing lipoproteins in the liver may exist, although no data are available to support this suggestion at present.

Lindholt et al and our laboratory have reported an inverse association of serum cystatin C levels with abdominal aortic aneurysm (AAA) progression,5,7 pointing to a role for cysteine proteases/cystatin C imbalance in aortic remodeling and expansion in humans. Systemic cystatin C deficiency in ApoE−/− mice recapitulated many features of human atherosclerotic aortic aneurysms, including severe medial elastic laminae degradation, tunica media thinning, and aortic ectasia,31 whereas cystatin C deficiency alone (Cyst C−/−ApoE−/−/− mice) did not display these phenotypes. These results indicate a combined effect of altered proteolysis (Figures 1 and 4) and cholesterol homeostasis and inflammation8 in the pathogenesis of aortic aneurysms. Typical atherosclerotic aortic aneurysms occur regionally accompanied by signs of inflammation and enhanced protease expression. The more diffuse aortic ectasia and elongation reported here likely reflect systemic deficiency of cystatin C with enhanced proteolytic capacity against extracellular matrix proteins throughout the aortae, suggesting a role for cystatin C in aneurysmal dilatation. TIMP-1−/−ApoE−/− mice showed similar findings.24,26,31 Deiciency of TIMP-1 led to increased medial degradation, macrophage infiltration, and thoracic-abdominal aneurysm formation. Therefore, both MMPs and cysteine proteases contribute to degradation of the medial elastica and aneurysm formation (Figure 4).24,26,31

In conclusion, these data in genetically altered mice establish that cystatin C maintains elastic lamina integrity in the arterial tunica media. The multiplicity of proteases involved in aortic aneurysm formation and atherosclerosis suggested by the ensemble of these results require consideration when contemplating antiprotease treatments in the clinic.

Acknowledgments

This study is supported by NIH grants HL67249 (to G.K.S.), HL60942, HL67283 (to G.P.S.), HL67204 (to H.A.C.), HL56985, and HL05698508 (to P.L.) and from American Heart Association Grant-in-Aid 0355130Y (to G.P.S.). We thank Eugenia Shvartz for technical assistance and Karen Williams for editorial assistance.

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Circ Res. 2005;96:368-375; originally published online January 13, 2005; doi: 10.1161/01.RES.0000155964.34150.F7

Circulation Research is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0009-7330. Online ISSN: 1524-4571

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