Enhanced Abdominal Aortic Aneurysm Formation in Thrombin-Activatable Procarboxypeptidase B–Deficient Mice


Objective—To determine whether procarboxypeptidase B (pCPB)−/− mice are susceptible to accelerated abdominal aortic aneurysm (AAA) development secondary to unregulated OPN-mediated mural inflammation in the absence of CPB inhibition.

Methods and Results—Thrombin/thrombomodulin cleaves thrombin-activatable pCPB or thrombin-activatable fibrinolysis inhibitor, activating CPB, which inhibits the generation of plasmin and inactivates proinflammatory mediators (complement C5a and thrombin-cleaved osteopontin [OPN]). Apolipoprotein E−/−OPN−/− mice are protected from experimental AAA formation. Murine AAAs were created via intra-aortic porcine pancreatic elastase (PPE) infusion. Increased mortality secondary to AAA rupture was observed in pCPB−/− mice at the standard PPE dose. At reduced doses of PPE, pCPB−/− mice developed larger AAAs than wild-type controls (1.01±0.27 versus 0.68±0.05 mm; P=0.02 [mean±SD]). C5−/− and OPN−/− mice were not protected against AAA development. Treatment with tranexamic acid inhibited plasmin generation and abrogated enhanced AAA progression in pCPB−/− mice.

Conclusion—This study establishes the role of CPB in experimental AAA disease, indicating that CPB has a broad anti-inflammatory role in vivo. Enhanced AAA formation in the PPE model is the result of increased plasmin generation, not unregulated C5a- or OPN-mediated mural inflammation. (Arterioscler Thromb Vasc Biol. 2010;30:1363-1370.)

Key Words: thrombosis • abdominal aortic aneurysm • carboxypeptidase • osteopontin • plasmin

Abdominal aortic aneurysm (AAA) is a common and potentially lethal disease, found in approximately 6% to 8% of men older than 60 years. AAA rupture is the most serious consequence of the disease and accounts for approximately 15,000 deaths in the United States annually.1,2 The pathogenesis of AAA disease is complex, involving chronic transmural inflammation, characterized by macrophage and lymphocyte infiltration and proteolytic degradation of medial elastin and interstitial collagens by matrix metalloproteinases (MMPs). Structural degradation of the aortic wall in combination with hemodynamic stress results in tissue failure and aneurysmal dilatation.3

Osteopontin (OPN) is a multifunctional protein that is both a component of the extracellular matrix (ECM) in mineralized tissues and a circulating proinflammatory cytokine.4 OPN is a prominent component of human atherosclerotic tissues and has recently been demonstrated to play a key role in AAA pathogenesis.5 Compared with apolipoprotein E (ApoE)−/− mice, ApoE−/−OPN−/− double-knockout mice are protected from angiotensin II–induced accelerated atherosclerosis and AAA formation.6 Structurally, OPN contains an RGD site (159–161) that mediates binding to a number of integrins, including αvβ1, αvβ3, αvβ5, and α5β1. Thrombin cleavage of OPN exposes a hitherto cryptic C-terminus (162SVVYGLR168) that mediates the binding of thrombin-cleaved OPN (OPN-R) to a new subset of integrins, consisting of α4β1 and α9β1.7–9 OPN-R demonstrates enhanced cell binding and cell migration compared with intact OPN in vitro,10,11 suggesting that thrombin cleavage of OPN may augment its proinflammatory properties.

When thrombin binds to thrombomodulin on the endothelial cell surface, it undergoes a remarkable change in its substrate recognition, switching from procoagulant factors, such as fibrinogen, to protein C and functionally inhibiting progressive thrombosis.12 In addition to inhibiting coagulation via the activation of protein C, the thrombin-thrombomodulin complex also influences fibrinolysis by activating a second physiological substrate, the plasma carboxypeptidase, thrombin-activatable procarboxypeptidase B (pCPB), or thrombin-activatable fibrinolysis inhibitor.13,14

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Activated carboxypeptidase B (CPB, or thrombin-activatable fibrinolysis inhibitor) cleaves C-terminal lysine or arginine, protecting partially digested fibrin clots from premature clot lysis.\textsuperscript{15} In addition to inhibiting fibrinolysis, thrombin-activated CPB has a broad substrate specificity; inactivating bradykinin, activated complement C5 (C5a), and OPN-R, converting OPN-R to an inactive form (OPN-desArg).\textsuperscript{16–19} In aggregate, these findings suggest that CPB may function in a feedback fashion to regulate thrombin’s inflammatory properties.

Given the importance of OPN in AAA pathophysiology, we hypothesized that pCPB\textsuperscript{7–/} mice would be susceptible to accelerated AAA progression secondary to unregulated OPN-R–mediated mural inflammation. Herein, we report enhanced aortic mural inflammation and AAA formation in pCPB\textsuperscript{7–/} mice. However, contrary to our hypothesis, the responsible mechanism is not unregulated OPN-R activity, but rather enhanced plasmin generation.

### Methods

#### Experimental AAA Creation

All animal work was reviewed and approved by the Laboratory Animal Care Committee at Stanford University. pCPB\textsuperscript{7–/} mice, originally created by George J. Broze, Jr., MD were backcrossed into C57Bl/6 mice for 10 generations. However, contrary to our hypothesis, the responsible mechanism is not unregulated OPN-R activity, but rather enhanced plasmin generation.

#### Tissue Preparation and Histological and Immunohistochemical Staining

Aortic tissues were processed and embedded in paraffin; blocks were sectioned at 4-μm thickness for hematoxylin-eosin, elastic Masson, and immunohistochemical staining, as previously described.\textsuperscript{20,21} For immunohistochemical staining, endogenous peroxidase activity was blocked with 0.3% hydrogen peroxide in methanol, and nonspecific binding sites were blocked with 10% normal serum (Dako Inc, Carpinteria, Calif). To stain for macrophages, sections were incubated with avidin-conjugated rat antimouse MAC-2 monoclonal antibody, 0.5 μg/mL (Cedarlane, Burlington, NC), followed by the appropriate biotinylated secondary antibodies (Dako Inc; and BioCare Medical, Concord, Calif). Appropriate isotypic antibodies (rabbit or rat IgG; Dako Inc) were used as negative controls.

### Histomorphometry

Histomorphometry was performed on Masson trichrome-stained histological sections, as previously described.\textsuperscript{20,21} The diameter of the lumen and the thickness of the medial layers were obtained from direct measurement of aortic sections; from these values, external aortic luminal diameters (EDs) were calculated. A “shrinkage index” (×1.25 for length) was used to correct for distortion caused by fixation and staining procedures. Macrophage counts were performed on 5-μm aortic cross-sections stained with MAC-2. Macrophage quantification was performed at the maximum luminal diameter. Cross-sections were divided into 4 quadrants (×200 magnification). MAC-2+ cells in each quadrant were counted. Mean and SD numbers of MAC-2+ cells per high-power field are reported.

### Ultra–High-Frequency Ultrasonography

Transabdominal 40-MHz B-mode ultrasonography was used to measure aortic diameter in vivo in selected mice, as previously described.\textsuperscript{23,24} Mice were imaged under inhaled anesthesia (2% isoflurane) with a commercially available system (Vevo 770 Imaging System; VisualSonics, Ontario, Toronto, Canada). The luminal diameter was measured at the sagittal plane before PPE infusion and on postinfusion days 7 and 14 using computer software (VisualSonics).

### Ex Vivo Clot Lysis Measurement

Mouse platelet-poor plasma was obtained by centrifugation of citrated blood. Platelet-poor plasma, 15 μL; rabbit thrombomodulin, 15 μL (American Diagnostica Inc, Stamford, Conn); and assay buffer, 60 μL (40-mmol/L Hepes and 150-mmol/L NaCl, pH 7.4) were premixed in a 96-well plate. Then, a mixture of 4 μL of thrombin (1 μmol/L), 2 μL of calcium chloride (1 mol/L) 8 μL of tissue plasminogen activator (tPA) (50 μg/mL) (EMD Chemicals Inc, Darmstadt, Germany), and 16 μL of water was added to the wells. Absorbance at 405 nm was kinetically measured at 37°C for 90 minutes. The clot lysis time is defined as the time at which the absorbance is half the difference between the plateau reached after clotting and the baseline value achieved at complete lysis.\textsuperscript{18}

### ELISA

Total MMP-2 and MMP-9 protein levels in aortic tissue lysates were measured using ELISA kits (R&D Systems, Minneapolis, Minn). Levels were normalized to total protein extracted from the aortic tissues. Plasma levels of tissue IPA, urokinase, and plasminogen were determined by ELISA (American Diagnostica Inc). Granulocyte macrophage colony-stimulating factor, interferon γ, interleukin (IL)-1β, IL-2, IL-4, IL-5, IL-6, IL-10, IL-12p70, and tumor necrosis factor α were measured in plasma using a 12-cytokine kit (Beadlyte; Millipore, Billerica, Mass) and a platform (Luminex xMAP 100IS; Austin, Tex).

### Statistical Analysis

Data are given as the mean±SD. \( \chi^2 \) and 2-tailed \( t \) tests were used for individual comparison of ordinal and nominal data. Two-way ANOVA with the Bonferroni/Dunn post hoc correction for nonnormally distributed populations (StatView Software; SAS, Cary, NC) was used for comparison of TA-treated pCPB\textsuperscript{7–/} and wild-type (WT) animals and untreated controls. Differences were considered statistically significant at \( P<0.05 \).

### Results

pCPB Deficiency Increases Susceptibility to PPE-Induced AAA Rupture

At the standard PPE concentration of 15 U/mL,\textsuperscript{21} pCPB\textsuperscript{7–/} mice (n=9) developed AAAs rapidly, with 66% of the animals experiencing a rupture by postinfusion day 3. Mortality secondary to AAA rupture is unusual in the PPE infusion model. No ruptures were observed in WT mice.
Figure 1. Comparison of pCPB\(^{−/−}\) and WT aortae after PPE infusion. A, AAA rupture rates at decreasing concentrations of PPE. B, Aortic diameter vs time after PPE, 7.5 U/mL, infusion. External aortic diameters at day 0 (pCPB\(^{−/−}\), n=5; WT, n=5), 3 (pCPB\(^{−/−}\), n=13; WT, n=11; \(*P<0.001\)), and 7 (pCPB\(^{−/−}\), n=8; WT, n=5; \(\dagger P<0.02\)) were determined by histomorphometry. C, Representative aortae 7 days after PPE infusion, 7.5 U/mL. Arrows indicate the origin of the left renal artery.

Enhanced AAA formation was observed in homozygous pCPB\(^{−/−}\) mice, but not in heterozygous pCPB\(^{−/+}\) mice, 7 days after PPE infusion. Full-strength elastase, 15 U/mL, resulted in aortic diameters of 0.76±0.24 mm in the pCPB heterozygotes (n=6). There was no significant difference between pCPB\(^{−/+}\) and WT (n=5, 0.73±0.09 mm) aortic diameters at 7 days (P=0.80). No ruptures were observed in heterozygous mice.

Elastic Masson staining of representative pCPB\(^{−/−}\) AAA sections revealed disruption of elastic fibers within the media as early as postinfusion day 3. In comparison, the WT aortic media and elastic laminae remained intact and well organized (Figure 2A). Seven days after PPE infusion, medial elastin in pCPB\(^{−/−}\) mice was circumferentially intact, with extensive fragmentation of the elastic laminae. Damage to the aortae of WT animals was less severe, with evidence of only mild disorganization and disruption of medial elastin (Figure 2B). Representative aortic sections from negative controls infused with saline demonstrated preservation of elastic lamellae within the aortic media, confirming that PPE is responsible for aneurysm induction (Figure 2C).

Mural inflammation, as quantified by number of macrophages per high-power field, was more severe in pCPB\(^{−/−}\) mice after PPE administration (n=4, 289±151) than in PPE-infused WT animals (n=5, 102±42) (P=0.03). Saline-infused WT mice (n=2) had significantly fewer mural macrophages (11±8) compared with PPE-infused mice. Intense transmural inflammation involving the media and adventitia was observed in pCPB\(^{−/−}\) AAs. WT aortae were characterized by extensive adventitial inflammation. However, little inflammatory infiltrate was observed within the media of WT aortae (Figure 2D).

**OPN and C5 Deficiencies Do Not Protect Against AAA Development**

To determine whether the augmented aortic inflammation and aneurysmal dilatation observed in pCPB\(^{−/−}\) mice was the result of unregulated OPN-R activity in the absence of CPB,
we infused OPN−/− mice with low-dose PPE, 7.5 U/mL. No significant difference between OPN−/− (n=8, 0.70±0.17 mm) and WT (n=5, 0.68±0.05 mm) (P=0.80) ED was observed 7 days after PPE infusion. More important, the reduced-dose PPE failed to promote aneurysmal dilatation in OPN−/− and WT animals. Therefore, the experiment was repeated at the standard PPE concentration, 15 U/mL. Seven days after infusion of standard-dose PPE, there was no significant difference between the ED of OPN−/− mice (n=5, 0.77±0.10 mm) and WT mice (n=5, 0.73±0.09 mm) (P=0.50). Although there was dilatation with comparable degenerative changes in OPN−/− and WT aortae (Figure 3A), neither cohort achieved a 50% increase in aortic diameter at the 7-day point. Using serial transabdominal ultrasonography, the study was repeated again and performed to postinfusion day 14. Consistent with the 7-day study comparing ED, OPN deficiency did not protect mice from aortic dilatation after administration of standard-dose PPE (Figure 3B). On postinfusion day 7, OPN−/− mice (n=3) and WT mice (n=6) had aortic diameters of 0.88±0.02 and 0.87±0.12 mm, respectively (P=0.90). Fourteen days after PPE infusion, the aortic diameter was greater in OPN−/− mice (n=3, 1.04±0.07 mm) than WT control mice (n=6, 0.99±0.10 mm) (P=0.12); however, this difference did not reach significance, indicating that OPN does not play a major role in the pathogenesis of PPE-induced AAAs. Considering baseline diameters, both OPN−/− and WT mice achieved aneurysmal diameters by day 14. No ruptures occurred in either cohort.

C5a is a potent chemoattractant and mediates a variety of inflammatory disorders.25 Like OPN-R, C5a is also inactivated by CPB.16,17 We hypothesized that unregulated C5a activity in the absence of CPB may be responsible for enhanced aneurysm formation after PPE infusion. However, rather than attenuated disease, C5−/− animals demonstrated severe aneurysmal degeneration (Figure 3A) and dilated EDs (n=3, 1.05±0.37 mm) 7 days after PPE, 15 U/mL, infusion. No ruptures were observed at the standard dose of PPE in C5−/− animals. This study was repeated, using serial transabdominal ultrasonography, to monitor aortic diameter, and performed to postinfusion day 14. There was no significant difference in aortic diameter between C5−/− mice (n=4,
0.92±0.12 mm) and WT mice (n=5, 0.93±0.09 mm) (P=0.70) on postinfusion day 7. However, C5\(^{-/-}\) animals (n=4) developed significantly larger AAAs than WT controls (n=5) by day 14 (1.59±0.68 versus 0.97±0.12 mm; P<0.001) (Figure 3C). These data suggest that neither OPN nor C5a dysregulation in the absence of CPB is responsible for enhancing AAA formation after PPE administration in pCPB\(^{-/-}\) animals.

Enhanced AAA Development Secondary to Increased Plasmin Generation Is Abrogated by TA Administration

CPB diminishes plasmin generation by cleaving C-terminal lysines from partially digested fibrin clots, reducing plasminogen and tPA incorporation into the fibrin clot and subsequently suppressing fibrinolysis.\(^{13-15}\) Although the major physiological role of plasmin is the degradation of fibrin polymers, it also mediates the activation of a number of proteases (ie, MMP-2 and MMP-9) involved in the aneurysmal degeneration of the infrarenal aorta.\(^{26-29}\) To determine whether enhanced AAA development in pCPB\(^{-/-}\) mice results from enhanced plasmin generation, we induced AAAs (PPE, 10 U/mL) in pCPB\(^{-/-}\) and WT mice treated with TA, a compound that inhibits plasmin generation, and monitored them with transabdominal ultrasonography in vivo. Treatment with TA significantly attenuated the aneurysmal response to PPE infusion in both pCPB\(^{-/-}\) (n=6, 0.91±0.09 mm; P<0.05) and WT (n=5, 0.87±0.03 mm; P<0.05) animals examined 7 days after aneurysm induction (Figure 4). As previously observed, pCPB\(^{-/-}\) animals (n=4) treated with saline after PPE, 10 U/mL, infusion developed significantly larger AAAs than saline-treated WT controls (n=6) (1.36±0.20 versus 1.12±0.07; P<0.05) by postinfusion day 7.

TA inhibition of plasmin generation significantly prolonged ex vivo clot lysis time in both pCPB\(^{-/-}\) (737.5±94.6 versus 237.5±53.0 seconds; P=0.003) and WT (1640.0±412.9 versus 910.0±65.2 seconds; P=0.005) animals when compared with saline-treated controls 7 days after aneurysm induction.
induction (Figure 4). Baseline clot lysis time was markedly shorter in saline-treated pCPB−/− mice than in similarly treated WT controls (237.5±53.0 versus 910.0±65.2 seconds), confirming the role of CPB as a fibrinolytic inhibitor. TA administration prolonged the time to lysis by nearly 3.0- and 1.8-fold in pCPB−/− and WT mice, respectively.

Plasma levels of tPA, urokinase, and plasminogen were measured via ELISA to further characterize the role of fibrinolytic mechanisms in experimental aeurysm pathology after PPE infusion. Importantly, the hematologic profile of pCPB−/− mice is well characterized. pCPB deficiency does not alter prothrombin time, activated partial thromboplastin time, plasma fibrinogen levels, or protein C activation. There was no statistically significant difference between pCPB−/− (n=5) and WT (n=5) mice in plasma levels of the following profibrinolytic factors: tPA, 2.89±1.34 versus 3.69±1.80 ng/mL (P=0.18); urokinase, 4.98±0.80 versus 5.61±1.89 ng/mL (P=0.25); and plasminogen, 190.40±114.00 versus 136.10±82.00 μg/mL (P=0.20).

To determine the effect of plasmin-promoted mural proteolysis in the absence of CPB regulation, we measured MMP-2, MMP-9, and MMP-13 activity levels via gelatin zymography. Pooled aortic samples from pCPB−/− and WT animals taken 7 days after aneurysm induction demonstrated marked, albeit statistically insignificant, increases in MMP-2, MMP-9, and MMP-13 activity when compared with control animals infused with saline (data not shown). These data appear to coincide with the observation that MMP activity is significantly upregulated after PPE-induced aortic injury and vital to the process of aneurysmal degradation.30 There was no difference in MMP activity between pCPB−/− and WT AAAs. Analysis of MMP-2 and MMP-9 antigen levels via ELISA demonstrated increases in both pCPB−/− and WT aortae after PPE infusion when compared with animals infused with saline, supporting observations regarding MMP activity determined by gelatin zymography. TA administration reduced MMP-2 and MMP-9 activity and antigen levels when examined via zymography and ELISA, respectively (data not shown).

Inflammatory and anti-inflammatory cytokine levels were measured in the plasma of TA- and saline-treated pCPB−/− and WT mice 7 days after PPE infusion. No significant differences between groups were found for granulocyte macrophage colony-stimulating factor, interferon γ, IL-1β, IL-2, IL-4, IL-5, IL-6, IL-10, IL-12p70, and tumor necrosis factor α (data not shown).

**Discussion**

We originally hypothesized that OPN-R plays a major role in mediating the proinflammatory properties of OPN in the pathogenesis of AAA and that pCPB−/− mice, as a result of their inability to convert active OPN-R to inactive OPN-desArg, would demonstrate a propensity for more severe mural inflammation and enhanced AAA development after PPE infusion. The expected outcome was well supported by previous studies demonstrating the importance of OPN in angiotensin II-induced experimental AAAs in ApoE−/− animals.6 The prediction that pCPB deficiency would promote the development of larger AAAs proved to be true (Figures 1 and 2). However, our experiments refuted our hypothesis that unregulated OPN activity in the absence of CPB is responsible for mural inflammation and aneurysmal dilatation in the PPE infusion model of AAA disease. OPN deficiency failed to protect mice from AAA development after local infusion of PPE (Figure 3), indicating that OPN is not a critical mediator of AAA formation in this model.

Intrigued by these findings, we turned our attention to additional proinflammatory factors regulated by CPB. Specifically, we investigated the role of C5a16,17 in the promotion of AAA formation. C5a is a potent chemoattractant known to enhance inflammation in the absence of CPB in a murine model of alveolitis.19 Like OPN, C5a has recently been shown to play an important role in experimental AAA development.31 C5 deficiency alone failed to protect mice from AAA development after PPE administration. We observed acceleration of aneurysm progression in the absence of C5(a) (Figure 3C). Previous work utilizing C5−/− mice in the study of acute pancreatitis indicates that in addition to their well-known roles as pro-inflammatory mediators, C5a and its receptor (C5aR) may also mediate anti-inflammatory effects.32 C5−/− animals developed more severe pancreatitis and associated lung injury after cholecystokinin analog (cerulein) administration versus C5-sufficient controls. Whether this mechanism is responsible for the accelerated AAA progression in C5−/− mice remains to be determined.

Thrombin-activatable pCPB downregulates the generation of plasmin and subsequently suppresses clot lysis.13–15 Plasmin is a serine protease with broad substrate recognition. In addition to fibrinolysis, plasmin activates multiple ECM-degrading proteinases, including MMP-2, MMP-3, MMP-9, MMP-12, and MMP-13,28 directly and indirectly, promoting dissolution of structural proteins within the aortic media. These MMPs not only destroy mural elastin and collagen but also mediate the mural infiltration of macrophages, promoting further inflammation and degradation.26 Indeed, mice deficient in plasminogen and urokinase and those overexpressing plasminogen activator inhibitor-1 (PAI-1) are protected from experimental AAA development.26,27,29

We demonstrated that inhibition of plasmin generation from plasminogen by TA attenuates AAA formation in pCPB−/− mice, indicating that unregulated plasmin generation in the absence of CPB within the infused aortic segment promotes inflammation and aneurysmal degeneration of the aorta (Figure 4). TA completely abolished AAA rupture in pCPB−/− animals after PPE, 10 U/mL, infusion. The efficacy of TA inhibition of plasmin generation was verified via ex vivo clot lysis analysis.

Enhanced local plasmin generation and MMP-2 and MMP-9 activation have been previously reported in the renal tissues of pCPB−/− mice using either an immune complex-mediated glomerulonephritis model or an obstructive nephropathy model.33,34 As expected, analysis of plasma and aortic tissue at day 7 by ELISA and gelatin zymography demonstrated increased levels and activity of MMP-2 and MMP-9 in both pCPB−/− and WT animals after PPE.
The role of plasmin in the promotion of AAA formation is likely not limited to the activation of MMPs. Although it does not participate in elastin and collagen dissolution directly, plasmin has the ability to degrade multiple ECM proteins, including laminin and fibronectin. In addition, plasmin may stimulate the infiltration of inflammatory cells at sites of tissue injury and may promote the activation of TGF-β1 and its release from matrix components. Enhanced plasmin generation may also lead to premature dissolution of fibrin, which serves as a provisional ECM for fibroblasts, thus impairing tissue repair. The results of our study are consistent with published evidence highlighting the pivotal role of plasmin in the pathogenesis of AAA. Taking this concept a step further, we demonstrated the importance of CPB in regulating plasmin generation and activity, especially regarding plasmin’s cleavage of nonfibrin substrates in AAA pathogenesis in vivo (Figure 5).

The failure of OPN deficiency to protect mice from aneurysmal dilatation after PPE infusion is not consistent with previous findings in ApoE−/− OPN−/− mice infused with angiotensin II. It is likely that this is the result of inherent differences between these 2 complementary but pathophysiologically distinct models. PPE infusion results in an acute injury, followed by immediate mononuclear cell infiltration and mural inflammation; these phenomena tend to develop on the order of days to weeks in the ApoE−/− angiotensin II model in association with hypertension, increasing atherosclerotic burden and microdissection of the aortic wall. We induced varying degrees of injury by reduced and standard PPE concentrations, examined aortae at different time points, and found no difference between OPN−/− mice and WT controls regarding aortic dilatation. All OPN−/− mice developed AAA, defined as a greater than 50% increase from baseline aortic diameter, after infusion of standard dose PPE. In comparison, in previous studies demonstrating the importance of OPN in AAA development, ApoE−/− OPN−/− mice infused with angiotensin II were nearly completely protected from aneurysmal dilatation. It is possible that OPN-mediated mechanisms of aneurysm formation are less important in the setting of severe acute inflammation after PPE administration. However, our findings do not diminish the importance of OPN in its capacity to promote vascular disease, specifically AAAs, in humans. Indeed, increased serum and aortic wall OPN levels have been demonstrated in patients with AAA disease. It would be interesting to determine whether pCPB deficiency would lead to enhanced AAA formation in the ApoE−/− angiotensin II model.

It was recently shown that CPB is capable of regulating the activity of both bradykinin and C5a in vivo. The current study, and other recent studies using pCPB−/− mice, expand CPB’s regulation to plasmin activity for nonfibrin substrates, further supporting the thesis that CPB has broad anti-inflammatory effects and may function as a homeostatic mechanism regulating thrombin’s proinflammatory properties in vivo. Highlighting the potential importance of fibrinolytic and thrombotic mechanisms in human AAA disease, serum-activated protein C–protein C inhibitor complex was recently identified as a biological marker for AAA; the rapid growth of the intraluminal thrombus was proposed to be a better predictor of aneurysm rupture than diameter itself. The study of the close interaction between thrombosis and inflammation will further illuminate the pathogenesis of this complex clinical disorder.

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Disclosures
None.

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In the article “Enhanced Abdominal Aortic Aneurysm Formation in Thrombin-Activatable Procarboxypeptidase B-Deficient Mice” by Schultz et al, which appeared in the July 2010 issue of the journal (Arterioscler Thromb Vasc Biol. 2010;30:1363–1370; DOI: 10.1161/ATVBAHA.109.202259), a footnote stating that Geoffrey Schultz and Maureen Tedesco contributed equally to the work should have been included on the first page of the article.

The online version has been corrected.

The publisher sincerely regrets the error.

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